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L3: Entry 2 of 2 File: EPAB Dec 9, 1993

PUB-NO: WO009324145A1
DOCUMENT-IDENTIFIER: WO 9324145 A1
TITLE: COMPOSITIONS USEFUL IN DIAGNOSIS AND PROPHYLAXIS OF LYME DISEASE

PUBN-DATE: December 9, 1993

INVENTOR-INFORMATION:

NAME	COUNTRY
GOLDE, WILLIAM T	US
ROEHRIG, JOHN T	US
BURKOT, THOMNAS	US
PIESMAN, JOSEPH F	US
JOHNSON, BARBARA J B	US
MAYER, LEONARD W	US
KEEN, MARK G	US
HUNT, ANN R	US

INT-CL (IPC): A61K 39/00; A61K 39/02; C12Q 1/00; C07K 3/00
EUR-CL (EPC): A61K039/02; C07K014/20, C07K016/12

ABSTRACT:

CHG DATE=19990617 STATUS=O>The present invention provides novel isolated B. burgdorferi antigens which have been regulated and differentiated in a tick vector. These antigens are useful in diagnosing Lyme disease and in compositions for prophylaxis thereof.

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L3: Entry 2 of 2 File: EPAB Dec 9, 1993

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INVENTOR-INFORMATION:

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ROEHRIG, JOHN T	US
BURKOT, THOMNAS	US
PIESMAN, JOSEPH F	US
JOHNSON, BARBARA J B	US
MAYER, LEONARD W	US
KEEN, MARK G	US
HUNT, ANN R	US

ASSIGNEE-INFORMATION:

NAME	COUNTRY
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US HEALTH	US
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KEEN MARK G	US
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APPL-NO: US09304984
APPL-DATE: May 2[illegible], 1993

PRIORITY-DATA: US[illegible]A May [illegible], 1992 , US[illegible]A September 14, 1992 , US[illegible]A December 1[illegible], 1992

INT-CL (IPC): A61K [illegible], A61K [illegible], [illegible]
EUR-CL (EPC): A61K039/02; C07K014/20; C07K016/12

ABSTRACT:

CHG DATE=19[illegible] STATUS O>The present invention provides novel isolated B. burgdorferi antigens which have been regulated and differentiated in a tick vector. These antigens are useful in diagnosing Lyme disease and in compositions for prophylaxis thereof.

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Search Results - Record(s) 1 through 13 of 13 returned.

1. Document ID: US 20020115112 A1

L7: Entry 1 of 13 File: PGPB Aug 22, 2002

PGPUB-DOCUMENT-NUMBER: 20020115112
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020115112 A1

TITLE: Neutrokine-alpha and Neutrokine-alpha splice variant

PUBLICATION-DATE: August 22, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Yu, Guo-Liang	Berkeley	CA	US	
Ebner, Reinhard	Gaithersburg	MD	US	
Ni, Jian	Germantown	MD	US	
Rosen, Craig A.	Laytonsville	MD	US	
Ullrich, Stephen	Rockville	MD	US	

US-CL-CURRENT: 435/7.2; 424/145.1, 530/388.23

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

2. Document ID: US 20020103122 A1

L7: Entry 2 of 13 File: PGPB Aug 1, 2002

PGPUB-DOCUMENT-NUMBER: 20020103122
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020103122 A1

TITLE: Methods of treatment and prevention of restenosis

PUBLICATION-DATE: August 1, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Rosen, Craig A.	Laytonsville	MD	US	
Ni, Jian	Germantown	MD	US	
Wang, Mingsheng	Flushing	NY	US	
Shi, Yuenian Eric	Roslyn Heights	NY	US	

US-CL-CURRENT: 514/12

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

3. Document ID: US 20020044941 A1

L7: Entry 3 of 13 File: PGPB Apr 18, 2002

PGPUB-DOCUMENT-NUMBER: 20020044941
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020044941 A1

TITLE: Nucleic acids, proteins and antibodies

PUBLICATION-DATE: April 18, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Rosen, Craig A.	Laytonsville	MD	US	
Ruben, Steven M.	Olney	MD	US	

US-CL-CURRENT: 424/184.1; 435/183, 435/320.1, 435/325, 435/6, 435/69.1, 435/7.1, 514/44, 536/23.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

4. Document ID: US 6403770 B1

L7: Entry 4 of 13 File: USPT Jun 11, 2002

US-PAT-NO: 6403770
DOCUMENT-IDENTIFIER: US 6403770 B1

TITLE: Antibodies to neutrokine-alpha

DATE-ISSUED: June 11, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Yu; Guo-Liang	Berkeley	CA		
Ebner; Reinhard	Gaithersburg	MD		
Ni; Jian	Rockville	MD		
Rosen; Craig A.	Laytonsville	MD		

US-CL-CURRENT: 530/387.3; 435/69.5, 435/7.1, 530/300, 530/324, 530/351, 530/388.1, 530/388.23

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

5. Document ID: US 6203798 B1

L7: Entry 5 of 13 File: USPT Mar 20, 2001

US-PAT-NO: 6203798
DOCUMENT-IDENTIFIER: US 6203798 B1

TITLE: Borrelia antigen

DATE-ISSUED: March 20, 2001

US-CL-CURRENT: 424/234.1; 424/184.1, 424/262.1, 435 7.2, 530/350

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Image

6. Document ID: US 6183986 B1

L7: Entry 6 of 13 File: USPT Feb 6, 2001

US-PAT-NO: 6183986
DOCUMENT-IDENTIFIER: US 6183986 B1

TITLE: OspA DNA and lyme disease vaccine

DATE-ISSUED: February 6, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bergstrom; Sven	Umea			SE
Barbour; Alan G	San Antonio	TX		
Magnarelli; Louis A.	Durham	CT		

US-CL-CURRENT: 435/69.1; 424/184.1, 424/234.1, 435/320.1, 435/6, 435/91.2, 536/23.4, 536/23.7

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Image

7. Document ID: US 6113914 A

L7: Entry 7 of 13 File: USPT Sep 5, 2000

US-PAT-NO: 6113914
DOCUMENT-IDENTIFIER: US 6113914 A

TITLE: Osp A proteins of Borrelia burgdorferi subgroups, encoding genes and vaccines

DATE-ISSUED: September 5, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Lobet; Yves	Rixensart			BE
Simon; Markus	Freiburg			DE
Schaible; Ulrich	Freiburg			DE
Wallich; Reinhard	Heidelberg			DE
Kramer; Michael	Freiburg			DE

US-CL-CURRENT: 424/234.1; 424/184.1, 424/185.1, 424/190.1, 424/283.1, 435/69.3, 435/7.32, 435/71.2, 436/543, 530/351, 530/387, 530/413, 530/806, 530/827, 530/825

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Image

DOCUMENT-IDENTIFIER: US 6083722 A

TITLE: Borrelia antigen

DATE-ISSUED: July 4, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bergstrom; Sven	Umea			SE
Barbour; Alan G.	San Antonio	TX		
Magnarelli, Louis A.	Durham	CT		

US-CL-CURRENT: 435/69.3; 435/6, 435/91.2, 536/23.4, 536/23.7

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | DWPI | Drawing Desc | Image

9. Document ID: US 5942236 A

L7: Entry 9 of 13 File: USPT Aug 24, 1999

US-PAT-NO: 5942236
DOCUMENT-IDENTIFIER: US 5942236 A

TITLE: Osp A proteins of Borrelia burgdorferi subgroups, encoding genes and vaccines

DATE-ISSUED: August 24, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Lobet; Yves	Rixensart			BE
Simon; Markus	Frieburg			DE
Schaible; Ulrich	Frieburg			DE
Wallich; Reinhard	Heidelberg			DE
Kramer; Michael	Frieburg			DE

US-CL-CURRENT: 424/234.1; 424/184.1, 424/185.1, 424/262.1, 435/69.3, 435/7.32, 436/543, 530/359, 530/403, 530/806, 530/820

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | DWPI | Drawing Desc | Image

10. Document ID: US 5688512 A

L7: Entry 10 of 13 File: USPT Nov 18, 1997

US-PAT-NO: 5688512
DOCUMENT-IDENTIFIER: US 5688512 A

TITLE: Borrelia antigen

DATE-ISSUED: November 18, 1997

INVENTOR-INFORMATION:

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | Draw Desc | Image

11. Document ID: US 5583038 A

L7: Entry 11 of 13 File: USPT Dec 10, 1996

US-PAT-NO: 5583038
DOCUMENT-IDENTIFIER: US 5583038 A

TITLE: Bacterial expression vectors containing DNA encoding secretion signals of lipoproteins

DATE-ISSUED: December 10, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Stover; Charles K.	Silver Spring	MD		

US-CL-CURRENT: 435/252.3; 424/93.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | Draw Desc | Image

12. Document ID: US 5582990 A

L7: Entry 12 of 13 File: USPT Dec 10, 1996

US-PAT-NO: 5582990
DOCUMENT-IDENTIFIER: US 5582990 A

TITLE: DNA encoding borrelia burgdorferi OspA and a method for diagnosing borrelia burgdorferi infection

DATE-ISSUED: December 10, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bergstrom; Sven	Umea			SE
Barbour; Alan G	San Antonio	TX		
Magnarelli; Louis A.	Durham	CT		

US-CL-CURRENT: 435/5; 435/32.1, 435/69.1, 530/350, 536/23.7

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | Draw Desc | Image

13. Document ID: US 5523089 A

L7: Entry 13 of 13 File: USPT Jun. 4, 1996

US-PAT-NO: 5523089
DOCUMENT-IDENTIFIER: US 5523089 A

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bergstrom; Sven	Umea			SE
Barbour; Alan G.	San Antonio	TX		
Magnarelli; Louis A.	Durham	CT		

US-CL-CURRENT: 424/262.1; 424/234.1, 435 7.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments

PDF | Claim Data | Image

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Term	Documents
P37.DWPI,EPAB,JPAB,USPT,PGPB.	658
P37S	0
FLAA.DWPI,EPAB,JPAB,USPT,PGPB.	53
FLAAS.DWPI,EPAB,JPAB,USPT,PGPB.	20
((FLAA OR P37) AND 6).USPT,PGPB,JPAB,EPAB,DWPI.	13
(L6 AND (P37 OR FLAA)).USPT,PGPB,JPAB,EPAB,DWPI.	13

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L7: Entry 6 of 15 File: USPT Feb 6, 2001

DOCUMENT-IDENTIFIER: US 6183986 B1
TITLE: OspA DNA and lyme disease vaccine

Brief Summary Text (2):
The present invention relates to immunogenically active fractions of Borrelia burgdorferi spirochaetes comprising antigenic polypeptides, proteins, glycolipids and carbohydrates useful for immunization against and diagnosis of Lyme disease, a method of preparing the immunogenically active fractions, a vaccine comprising an immunogenically effective amount of one or several of the immunologically active fractions or a part thereof, a diagnostic agent comprising one or several of the immunogenically active fractions or a part thereof, a DNA fragment encoding an antigenic polypeptide related to the outer membrane protein OspA present in the immunogenically active fractions, a monoclonal or polyclonal antibody directed against one or several of the immunogenically active fractions or antigenic polypeptide, and the use of the fractions, polypeptide or antibody for diagnostic and therapeutic purposes.

Brief Summary Text (4):
Lyme disease is a zoonosis caused by the tick-borne spirochaete B. burgdorferi (1). When a susceptible host is bitten by an Ixodid tick, B. burgdorferi organisms enter the skin. In humans the initial skin manifestation is termed erythema chronicum migrans (ECM) whereas a long-standing infection of the skin produces acrodermatitis chronica atrophicans (2). The Borrelia organisms also enter the circulatory system of the host and are distributed to various organs, including the brain and joints (3). A secondary spread of the pathogens produces a variety of clinical syndromes, including lymphocytic meningoradiculitis (4), myocarditis (5) and chronic arthritis (6). In many patients the infection of some tissues, particularly the brain and joints, persists for years and can be severely disabling. These forms of chronic Lyme disease are a consequence of the host's inability to rid itself of the infectious agent and perhaps the development of an autoimmune reaction (7).

Brief Summary Text (5):
Diagnosis of Lyme disease has chiefly been based on clinical evidence. The best marker during the primary stage of infection has been the presence of erythema chronicum migrans (ECM) but these skin lesions may not always develop or they may manifest atypically (7). Moreover, lyme disease can be confused with other illnesses characterized by neurological or arthritic manifestations. When clinical histories are incomplete, serologic testing with determination of antibody titers is the best laboratory method of diagnosis. Indirect fluorescent antibody (IFA) staining tests and enzyme-linked immunosorbent assays (ELISA) are used to detect total immunoglobulins (Ig) or class-specific IgM and IgG antibodies to B. burgdorferi (8). ELISA is usually preferred because the procedures are more easily standardized and automated and because absorbance values can be statistically analyzed to give more objective results (9).

Brief Summary Text (8):
Conventional diagnostic tests for Lyme disease have used whole spirochetal sonic extracts as test antigens in ELISA to detect antibodies to B. burgdorferi, but this test yields unsatisfactory low diagnostic sensitivity (10 to 40%) during the early

sclerosis, serum negative rheumatoid arthritis, juvenile rheumatoid arthritis, and Reiter's syndrome (9).

Brief Summary Text (9):
Several researchers have focused on isolating flagellin or preparing flagellin-enriched whole cell or fractions for diagnostic agents so as to improve diagnostic tests for an early diagnosis of Lyme disease. For this purpose, Coleman et al. (15) have obtained B. burgdorferi fractions by treating whole spirochaetes with the denaturating detergent sodium dodecyl sulfate (SDS) so as to obtain a protoplasmic cylinder flagellar (PC) fraction which upon subsequent shearing, filtration and dialysis constituted a flagellin-enriched fraction from which immunogenic polypeptides (flagellin) were eluted and used as antigens in ELISA for IgG and IgM antibodies. The flagellin-enriched fraction was reported to be a useful antigen for early stage reactivity. Also, Grodzicki et al. (58) discloses fractions of B. burgdorferi containing flagellin.

Brief Summary Text (10):
Hansen et al. (16) describes a method of preparing purified preparation of flagella usable as an antigen in an ELISA analysis for IgM antibody detection.

Brief Summary Text (11):
U.S. Pat. No. 4,721,617 discloses the use of inactivated whole B. burgdorferi spirochaetes as a vaccine against Lyme disease and broadly teaches the concept of using an outer envelope fraction or its component polypeptides in vaccines but does not distinguish or give guidance as to which components to select for this purpose.

Brief Summary Text (12):
EP 252 641 discloses the use of antibodies specific to one or more antigens of B. burgdorferi, e.g. related to the cell wall or cell membrane of the organism. OspA and OspB are mentioned as examples of such antigens and fractions of B. burgdorferi are mentioned in general. The antibodies are stated to be useful in detecting B. burgdorferi antigens in urine and in diagnosing Lyme disease.

Brief Summary Text (13):
As explained above, the enzyme-linked immunosorbent assays for the diagnosis of Lyme borreliosis have been based on whole cell preparations. Such ELISA methods have shown good sensitivity, but lacked specificity (8, 9 and 59). Other antigenic preparations have been used such as the flagellin and fractionated antigens containing flagellin (15 and 58). These tests have showed a sensitivity almost as good as the test based on whole cell antigens, and greater specificity. However, these latter tests have proved most useful in the diagnosis of early stages of Lyme disease. Flagellin or fractions containing flagellin has been shown to be less suitable for use in the diagnosis of later stages of Lyme disease, because of a low specificity, i.e. a high cross-reactivity with antibodies raised in connection with other related diseases. The specificity of an assay for B. burgdorferi antibodies of various stages of Lyme disease, in which assay flagellin or a flagellin-enriched fraction is used, could be too low to be generally usable. Thus, there is a need for developing an assay for use in the diagnosis of various stages of Lyme disease which assay has a high sensitivity and specificity for B. burgdorferi antigens.

Brief Summary Text (14):
Furthermore, it would be desirable to provide individuals such as humans and animals with a broad protection against Lyme disease by means of immunization. The present invention discloses easily extracted immunologically active B. burgdorferi fractions that increase the specificity of assays for B. burgdorferi antibody and are potential vaccine components and useful in antibody tests for the immunization and diagnosis of Lyme disease.

Brief Summary Text (21):
From the above general explanation of the background of the invention it is evident that efforts have been focused on isolating antigens or fractions of B. burgdorferi which are useful in diagnosing Lyme disease. Various techniques for preparing fractions

diagnosed with a high specificity.

Brief Summary Text (22):
Fractions B, C and E of the present invention are novel. The method outlined above by which the fractions of the invention may be obtained involves several steps, which will be described in details below. One step in the method of obtaining the fractions of B. burgdorferi is the initial lysis of the B. burgdorferi spirochaetes. The lysis is performed under conditions which ensure that the outer membrane and the components attached thereto are substantially released from the cell wall and flagellar components whereby fractions of important antigenic components, which are valuable for late stage detection of Lyme disease, are obtained. These conditions may be fulfilled by use of a mild non-denaturating detergent which, as will be described below, is preferably a non-denaturing, water-dialysable lysating agent such as a non-ionic, zwitterionic or anionic detergent, e.g. octyl-.beta.-D-glucopyranoside (OGP). Since the fractions of the invention are substantially free from flagellar proteins, there is minimal cross-reactivity with antibodies directed against flagella from other bacteria.

Brief Summary Text (28):
In a further aspect, the present invention relates to immunologically active fractions of a B. burgdorferi spirochaete strain substantially identical to the immunologically active fractions B, C and E obtained when subjecting the same strain of B. burgdorferi spirochaetes to the procedure described in Example 1 as determined by methods of determining substantial identity. Examples of such methods of determining substantial identity are comparison of the protein distribution pattern as obtained by SDS-PAGE analysis and immunological methods, e.g. such as parallel ELISA in which the reactivity of sera with antigens is measured.

Brief Summary Text (30):
In a further aspect, the present invention relates to an immunologically active fraction of B. burgdorferi, preferably fraction B of B. burgdorferi, having substantially the same reactivity with sera from patients with Lyme disease as that of whole cells of B. burgdorferi, but with substantially less reactivity with sera from syphilitic patients. Because members of the Borrelia genus show common antigens with one another and with the treponemes (12)(13) the problem of immunologic cross-reactivity arises when using whole cell preparations in serologic tests. As shown in Table 2 of Example 1, comparable analyses for class-specific IgG antibody have revealed that fraction B shows comparable sensitivity and greater specificity than the whole cell preparation. Furthermore, cross-reactivity with treponemal antibodies is minimal.

Brief Summary Text (31):
Furthermore, in Example 1 it is shown that only 3 of 16 samples from syphilis patients were positive. In Example 5 it is shown that only 1 of 13 mononucleosis patients and 2 out of 70 Anti nuclear antibody (ANA) sera exceeded the cutoff value, i.e. were positive. These results show that use of this fraction, i.e. fraction B, in efforts to detect IgG antibody reduces the number of false positive reactions associated with immune responses to other treponemes. Normally when using whole cells of B. burgdorferi for diagnostic purposes, both a serological test for Lyme disease and a diagnostic test for syphilis are required in order to be able to determine the false positive signals and arrive at the desired, correct diagnosis. This complex and time consuming diagnosis method is especially necessary when employing flagellin-enriched whole cell diagnostic agents or diagnostic agents mainly comprising flagellin as the antigenic component. By use of fraction B of the present invention for diagnosis of Lyme disease, only one test is required, namely the serological test. Thus, fraction B of the present invention constitutes a very important and novel tool in the fast and accurate diagnosis of Lyme disease.

Brief Summary Text (32):
Fraction B reacts with a substantial percentage of the sera from patients with Lyme disease, e.g. at least about [illegible] of the sera from patients with Lyme disease. More preferably, fraction B reacts with at least [illegible] of the sera from patients with Lyme

Brief Summary Text

The substantial lack of cell wall and flagellar components is, as explained above, believed to be one of the reasons for the very advantageous diagnostic properties of the fractions of the invention.

Brief Summary Text (43):
As shown in FIG. 2, the profiles of Coomassie blue-stained proteins and of whole cell and fractionated lysates B, C and E of B. burgdorferi differ. When compared with the molecular weight standards, the stained gel reveals the surface proteins of 31 and 34 kd (OspA and OspB) in fractions B, E and in the whole cell lysate of B. burgdorferi strain 2591. The presence of OspA in these preparations has been verified by immunoblotting with monoclonal antibody H5332. Likewise, the presence of OspB in the preparations has been verified by immunoblotting with monoclonal antibodies H6831 and H6TS. In fraction C OspA and OspB were absent. The 41 kd protein of flagellin was absent in all three fractions B, C and E. Thus, the 41 kd protein of fraction E stated above did not react with the anti-flagellin monoclonal antibody H9724 in an ELISA and does not react with fractions B and E and does therefore not seem to be flagellin or a related protein. Fraction B also contains other major proteins with apparent molecular weights of 20, 21, 28, 38, 59, 66, 69 and 85 kd. The 38 kd protein did not react with monoclonal antibody H9724, showing that this is not the same as the flagellin antigen. Fraction C contains two proteins with molecular weights of about 40 kd and about 70 kd, respectively. Four proteins in fraction B may prove to be of particular interest, namely the 21, 59, 66 and 69 kd proteins. Antibodies against the 69 and 59 kd proteins have been found in sera from patients with Lyme disease, and these proteins may therefore be important in the B. burgdorferi infection, and be potential candidates for vaccine and diagnostic agent constituents in immunization and diagnosis of Lyme disease. The 66 kd protein is believed to be cleaved to a smaller size when whole cells of B. burgdorferi are incubated with proteases such as trypsin and proteinase K.

Brief Summary Text (97):
The term "functional equivalent" is intended to include all immunogenically active substances with the ability of evoking an immune response in animals, including humans, to which the equivalent polypeptide has been administered, e.g. as a constituent of a vaccine or a diagnostic agent, which immune response is similar to the immune response evoked by the OspA protein. Thus, equivalent polypeptides are polypeptides capable of conferring immunity to Lyme diseases.

Brief Summary Text (109):
The production of OspA or a part thereof by recombinant techniques has a number of advantages: it is possible to produce OspA or part thereof by culturing non-pathogenic organisms or other organisms which do not affect the immunological properties of OspA or part thereof, it is possible to produce OspA in higher quantities than those obtained when recovering OspA from any of the above described fractions B, C and E, and it is possible to produce parts of OspA which may not be isolated from B. burgdorferi strains. The higher quantities of OspA or parts thereof may for instance be obtained by using high copy number vectors for cloning the DNA fragment of the invention or by using a strong promoter to induce a higher level of expression than the expression level obtained with the promoters P1 and P2 present on the DNA fragment of the invention. By use of recombinant DNA techniques for producing OspA or parts thereof, unlimited amounts of a substantially pure protein or polypeptide which is not "contaminated" with other components which are normally present in B. burgdorferi isolates may be obtained. Thus, it is possible to obtain a substantially pure OspA protein, i.e. OspA which is not admixed with other B. burgdorferi proteins which have an adverse effect when present in a vaccine or a diagnostic agent in which the OspA is an intended constituent. A substantially pure OspA protein or a polypeptide part thereof has the additional advantage that the exact concentration thereof in a given vaccine preparation is known so that an exact dosage may be administered to the individual to be immunized.

Brief Summary Text (111):
An important aspect of the present invention is a vaccine for the immunization of a mammal, including a human being, against Lyme disease, which vaccine comprises an

periodically administer the vaccine described above to individuals subjected to contact with ticks bearing B. burgdorferi. It is contemplated that vaccination once a year such as in the springtime will provide a suitable protection of individuals in risk of B. burgdorferi infection. A suitable dose of immunogenic components for such a vaccination is 5-500 .mu.g. However, also more irregular immunizations may be advantageous, and any immunization route which may be contemplated or shown to produce an appropriate immune response can be employed in accordance with the principle of the present invention. Suitable administration forms of the vaccine of the invention are oral administration forms, e.g. tablets, granules or capsules, subcutaneous, intracutaneous or intramuscular administration forms or forms suitable for nasal or rectal administration.

Brief Summary Text (113):
As stated above, recombinant DNA technologies are useful for the preparation of diagnostic reagents and vaccines. Routine methods for vaccine production involve risks of obtaining unwanted side effects, e.g. due to the vaccine containing unwanted (or even unidentified) contaminants. An alternative approach to the production of new vaccines involves the insertion of one or more DNA sequences constituting one or more parts of the DNA sequence shown in FIG. 5 or parts thereof into a virus genome, e.g. into a retrovirus, vaccinia virus or Epstein-Barr virus genome, to produce a polyvalent vaccine. An especially interesting virus for the present purpose is vaccinia. Also, synthetic polypeptides which have been prepared by conventional methods, e.g. by solid or liquid phase peptide synthesis, are suitable for vaccines.

Brief Summary Text (114):
In a further aspect, the present invention relates to a non-pathogenic microorganism which carries and is capable of expressing an inserted nucleotide sequence which is the nucleotide sequence shown in FIG. 5 or part thereof for use as a live vaccine for the immunization of an animal against Lyme disease. For instance, the use of a live vaccine might be advantageous since it is presumed that vaccines based on living organisms show an excellent immunogenicity, and it is also contemplated that the use of a live vaccine will confer a lifelong immunity against Lyme disease so that repeated vaccination will not be needed.

Brief Summary Text (118):
As is explained above, fractions of B. burgdorferi spirochaetes selected from fractions B, C and E defined above or polypeptides encoded by the DNA sequence shown in FIG. 5 or parts thereof are useful in immunization against Lyme disease and in the preparation of a composition for the immunization against Lyme disease, i.e. as vaccine components.

Brief Summary Text (119):
In a further important aspect, the present invention relates to a diagnostic agent for the detection of B. burgdorferi antibodies in a sample, which agent comprises one or more fractions of B. burgdorferi spirochaetes selected from fractions B, C and E defined above. Further, the present invention relates to a diagnostic agent for the detection of B. burgdorferi antibodies in a sample, which agent comprises one or more polypeptides encoded by the DNA fragment shown in FIG. 5 or part thereof, or one or more of the proteins contained in any of the fractions B, C and E defined above or a combination of one or more of the polypeptides encoded by the DNA fragment or parts thereof and the proteins of the fractions.

Brief Summary Text (120):
As explained above, B. burgdorferi strains of different geographical origin differ in their protein profiles as judged by Coomassie staining of PAGE gels (cf. Examples 1 and 9). Thus, the pattern of antibody responses, besides being dependent on the stage of infection, may vary between individuals from different parts of the world. Therefore, it may prove advantageous to use a mixture of two or more fractions isolated from different B. burgdorferi strains in a diagnostic agent to be used in various parts of the world. For instance, the use of one fraction of european origin and one of american origin, e.g. a fraction B from each origin, may provide a diagnostic agent which allows detection of Borrelia specific antibodies of these geographical origins. If the

immunologically active fractions or components may be used as diagnostic reagents for the determination of the presence of B. burgdorferi. As will be apparent to a person skilled in the art, several techniques may be applied in connection with such diagnostic reagents. Thus, preferred embodiments of the invention are based on immunological reactions between antigens and antibodies, detection of said reaction and correlating the results obtained with results from reference reactions. Preferred assays of the invention are enzyme immunosorbent assays such as enzyme linked immunosorbent assays (ELISA), radio immuno assays (RIA), immuno electrophoresis assays and the like.

Brief Summary Text (132):
The ELISA and RIA methods are well established and may be carried out with existing laboratory equipment and may also be subjected to automation. The methods of the invention therefore have wide applicability in clinical laboratories for diagnostic purposes and for monitoring the results of vaccination procedures, and in the pharmaceutical industry as an assay for immunogens to be used in the production of vaccines.

Brief Summary Text (135):
Although in some cases such as when the diagnostic agent is to be employed in an agglutination assay in which solid particles to which the antigen is coupled agglutinate in the presence of a B. burgdorferi antibody in the sample subjected to testing, no labelling of the monoclonal antibody is necessary, it is preferred for most purposes to provide the antibody with a label in order to detect bound antibody. In a double antibody ("sandwich") assay, at least one of the antibodies may be provided with a label.

Brief Summary Text (144):
In an embodiment of the invention, the diagnostic agent may comprise an immunologically active component of B. burgdorferi which is coupled to a bridging molecule coupled to a solid support. The bridging molecule, which is designed to link the solid support and the immunologically active components may be hydrazide, Protein A, glutaraldehyde, carbodiimide, or lysine.

Brief Summary Text (145):
The solid support employed in the diagnostic agent of the invention is e.g. a polymer or it may be a matrix coated with a polymer. The matrix may be of any suitable solid material, e.g. glass, paper or plastic. The polymer may be a plastic, cellulose such as specially treated paper, nitrocellulose paper or cyanogenbromide-activated paper. Examples of suitable plastics are latex, a polystyrene, polyvinylchloride, polyurethane, polyacrylamide, polyvinylacetate and any suitable copolymer thereof. Examples of silicone polymers include siloxane.

Brief Summary Text (152):
The detection of B. burgdorferi antigens in a sample may be carried out by using some of the well known ELISA principles, e.g. direct, catching, competitive and double enzyme linked immunosorbent assay. In e.g. an inhibition assay a purified polypeptide preparation of the invention is attached to a solid support (e.g. a polystyrene microtiter tray); the test solution to be measured is mixed with specific reference antibodies, e.g. the antibodies of the present invention, and this mixture is incubated with the solid support provided with the polypeptide preparation as mentioned above. After sufficient washing, enzyme labelled antibodies are added, and finally enzyme substrate is applied. For further detailed information of the principles employed in ELISA techniques, see for instance Voller et al., 1978, [illegible]

Brief Summary Text (156):
In a further aspect, the present invention relates to a diagnostic agent for the detection of B. burgdorferi infection in humans and animals, which diagnostic agent comprises a DNA sequence which is homologous to a DNA sequence encoding an immunologically active component of B. burgdorferi.

[illegible]

Drawing Description Text [illegible]

FIG. 14 shows the IgG antibody responses to the B. burgdorferi B fraction ELISA in sera from 52 patients with early and late stage Lyme borreliosis. Control sera from 64 healthy individuals were also measured. The cutoff value, calculated from the 64 healthy control sera, is marked by a dotted line in the figure. The experiments leading to the results shown in the figure are described in further detail in Example 5.

Detailed Description Text (13):
Two liters of BSK II medium containing approximately 10.sup.11 cells of B. burgdorferi ATCC 35210) in late log phase of growth were harvested by centrifugation in a high speed Beckman J221 centrifuge at 9,000.times. g for 20 minutes at 20.degree. C. and washed once with TSM buffer. The resulting pellet was resuspended in 10 ml of TSM buffer and placed on ice. After 15 minutes, 2.4 ml of 10% octyl-.beta.-D-glucopyranoside (OGP; Calbiochem, San Diego, Calif.) in TSEA were added. The cell suspension was incubated at 37.degree. C. for 1 hour. The resultant cell lysate was centrifuged at 48,000.times. g for 30 minutes at 25.degree. C. A clear OGP supernatant (S37) and an OGP-insoluble white pellet (P37) were obtained. The supernatant was then incubated at 56.degree. C. for 30 minutes. The flocculent white precipitate (P56) formed after the heating was separated from soluble constituents (S56) by centrifugation at 48,000.times. g for 30 minutes at 37.degree. C. The original pellet (P37) was washed by resuspension in 10 ml of TSEA, centrifuged at 48,000.times. g for 5 minutes and suspended in 10 ml of 1% sodium lauryl sarcosinate (Sarkosyl) in TSEA and incubated at 37.degree. C. for 1 hour and then at 20 degree. C. for 15 hours. The P56 fraction was treated in the same way as P37. The P37 suspension remained opalescent, while the P56 fraction cleared when incubated in Sarkosyl. Both fractions were centrifuged at 48,000.times. g for 30 minutes at 25.degree. C. There was a large translucent Sarkosyl-insoluble pellet (P37-p) and a clear supernatant (P37-s) in the P37 tube. In the P56 tube, there was no discernible pellet; only the supernatant was saved. The P37-s and P56-s fractions were each dialyzed against 25% methanol in glass-distilled water at 20.degree. C. The contents of the dialysis bags (Bethesda Research Laboratories) were lyophilized, and P37-s and P56-s fractions that were recovered were designated fraction F and fraction E, respectively. Fraction S56 was passed through a 0.45 micron nitrocellulose filter (Millipore low protein binding filter) and then dialyzed against glass-distilled water at 4.degree. C. The S56 precipitate that formed in the dialysis bag was recovered by centrifugation (48,000.times. g for 30 minutes at 25.degree. C.). The water-insoluble pellet was designated fraction B and the water-soluble supernatant was designated fraction C. Both fractions were lyophilized. Fraction P37-p was resuspended in 10 ml of 1% Sarkosyl in TSEA and incubated for 1 hour at 37.degree. C. This suspension was then centrifuged at 48,000.times. g for 30 minutes at 25.degree. C. The supernatant was discarded. The pellet was resuspended in 2% SDS in TSEA and incubated at 65.degree. C. for 30 minutes. The suspension was then centrifuged (48,000.times. g for 30 minutes at 25.degree. C.). The pellet was designated fraction A and was washed in glass-distilled water, whereas the supernatant (designated fraction D) was dialyzed against 25% methanol. Both fractions were lyophilized. There were insufficient amounts of fraction A produced for extensive testing. This fraction was therefore not used.

Detailed Description Text (17):
Serologic Test Enzyme-Linked Immunosorbent Assay (ELISA)

Detailed Description Text (18):
Two isolates of B. burgdorferi, the Shelter Island, New York strain, B31 ATCC [illegible] and a Connecticut strain, [illegible] white footed mouse, Anderson et al., [illegible], were maintained in BSK II medium. Fractionated preparations of spirochaetes were derived from stocks of the B31 strain, while whole cells used in ELISA were taken from subcultures of the Connecticut strain.

Detailed Description Text (19):
Serum samples from persons who had Lyme disease, relapsing fever, yaws, or syphilis were tested against the whole cell or fractions of B. burgdorferi in ELISA. The test procedures were essentially as described by Miller et al. [illegible]

well) to 96-well, flat-bottomed, polystyrene plates (Nunc, Denmark). The positive control sera were from persons who had erythema migrans and who lived in areas endemic for Lyme disease. After incubation for 18 to 20 hours at 37.degree. C. (at which time the wells were dry), 200 .mu.l of 0.5% donor horse serum in PBS were added to each well to block binding sites not covered with antigen. Plates were incubated for 1 hour at 37.degree. C. and washed three times with PBS-0.05% Tween 20.

Detailed Description Text (16):
To determine critical regions for positive test results, normal human serum specimens were screened against fractionated (n=22-27 sera tested) and whole cell preparations of B. burgdorferi (n=78 sera). The screening was performed for total immunoglobulins and IgG. The results are listed in Table 1. Average net absorption values for samples tested against the fractions ranged from 0.20 to 0.25 and from 0.18 to 0.23 for serum dilutions of 1:320 and 1:640, respectively. In ELISA with whole cell B. burgdorferi, cut-off values of 0.26 and 0.17 were recorded. Net absorption values for the positive control sera were usually considerably higher than those listed above, regardless of the antigen used.

Detailed Description Text (17):
Comparative analyses for class-specific IgG antibody revealed differences in specificity and sensitivity when sera were tested with the fractions. The results are listed in Table 2. For example, of the 22 serum specimens from persons who had relapsing fever, yaws, or syphilis that reacted positively to whole cells of B. burgdorferi, 7 (32%) remained reactive to fraction B. Only three of 16 samples from patients with syphilis or yaws were positive. In contrast, 30 (91%) of 33 specimens from patients who had Lyme disease and homologous antibody to whole cells of B. burgdorferi reacted positively to fraction B. The 3 samples that did not react to fraction B had relatively low antibody titers (1:640-1:1280) when tested against whole cells. Greater losses of sensitivity were noted in tests with the other fractions.

Detailed Description Text (18):
Serum specimens that were reactive in assays with whole cells of B. burgdorferi were reanalyzed in class-specific ELISA with the fractions of B. burgdorferi to determine the variability of titration end points. The results are shown in Table 3. Titers for 28 sera differed by 2 fold or less (n=15 samples) or by 4 fold (n.ltoreq.11) when fraction B was coated to the solid phase. Titers for the other two samples differed by 8 fold. Titration end points for 15 samples were usually higher in assays with fraction B than with whole cells. In tests for reproducibility, antibody titers to fraction B differed by 2 fold or less (n=13 samples), 4 fold (n=1), or by 8 fold (n=1) in the second trial. All 12 negative sera were likewise non-reactive in duplicate tests. When results for fractions C, D, E, and F were compared to those of whole cell or to fraction B reactivity (Table 3), 8 or 9 sera were considered positive, respectively. Antibody titers varied by as much as 32 fold.

Detailed Description Text (26):
The procedure outlined in Example I for preparing cell fractions was repeated with different detergents in step a), comprising the lysing of B. burgdorferi spirochaete cells. Fraction B was analyzed for activity in ELISA with different samples of patient serum. The different detergents tested were Zwittergent 3-10 and decyl-.beta.-D-glycopyranoside compared with SDS. No differences in reactivity against patient sera in ELISA could be seen with Fraction B prepared with the three different detergents. The results obtained appear from the following table

Detailed Description Text (27):
The experiments were carried out substantially as described in Example 1 above in the section "Serologic test enzyme-linked immunosorbent assay (ELISA)".

Detailed Description Text (34):
Serum samples were obtained from 30 patients with erythema chronicum migrans. The clinical symptoms of these patients and the serologic results of the sera in an ELISA based on whole cell antigen have previously been published (2 . . 22 patient sera obtained from clinically

sera from patients with serologically verified syphilis were also tested.

Detailed Description Text (85):
ELISA Methodology

Detailed Description Text (88):
The sera included as negative controls showed little reactivity in the ELISA. The cutoff value was 0.11 using a serum dilution of 1:500. This may partly be due to the antigen used, but may also depend on little exposure of individuals in Northern Sweden to B. burgdorferi. The use of these individuals shows that cross-reactivity to the antigens in fraction B seems to be negligible. Despite this, the IgG antibody responses of previously Borrelia-infected individuals were similar to those recorded in tests with whole cell B. burgdorferi. Thus, this fraction seems appropriate for serologic confirmation of the later stages of Lyme borreliosis. In the ELISA, forty positive Lyme borreliosis sera (30 low titer and 22 high titer sera) were assayed. The cutoff value in these tests was defined as the mean plus three standard deviations ([SD.times.3]+x) for sera from 64 persons from Northern Sweden with no known exposure to Lyme borreliosis. The results are seen in Table 4. Fraction B exhibited the highest sensitivity and specificity in this ELISA. Fraction B was further assayed with sera from persons who had had other diseases; these results are shown in Table 5. Ten sera from patients with reactive arthritis and serum specimens which had shown reactivity in tests for rheumatoid factor (49 sera), and Wassermann (10 sera) did not give any significant net absorption in the ELISA based on the B antigen. Among the sera displaying anti-nuclear activity (ANA-positive sera), two out of 70 (3%) had a net absorption above the cutoff value. One out of 13 (15%) sera from mononucleosis patients exceeded the cutoff value in the ELISA.

Detailed Description Text (89):
Out of 30 sera from early borreliosis, 13 (43%) had a net absorption above the cutoff value in the B-ELISA. All high titer sera had a net absorption greater than the cutoff value.

Detailed Description Text (129):
38. Bergstrom, S. et al., Molecular analysis of linear plasmid encoded major surface proteins OspA and OspB, of the Lyme disease spirochaete Borrelia burgdorferi, Molecule Microbiology (1989) 3(4), 479-486.

Detailed Description Text (143):
53. Voller et al., The Enzyme Linked Immunosorbent Assay (ELISA), 1979, Dynatech Europe, Borough House, Guernsey.

Detailed Description Text (150):
59. Shresta, M., Grodzicki, R. L., and Steere, A. C.: Diagnosing early Lyme disease. Am. J. Med., 1985, 78: 235-240.

Detailed Description Paragraph Table (1):
TABLE 1 Reactivity of normal human serum samples with whole cells or frac- tions of B. burgdorferi in ELISA Total immunoglobulins IgG Prote- Critical regi- Critical regi- in con- Total ons.sup.a for serum Total ons.sup.a for serum Anti- tents sera dilutions of sera dilutions of gens (mu.g/ml.sup.b tested 1:32 .sup.c 1:64 .sup.c tested 1:32 .sup.c 1:640.sup.c Whole- 95 28 0.26 0.17 [illegible] 0.18 0.13 cell Fraction: B 1[illegible] [illegible] 0.21 [illegible] 27 [illegible] .sup.a 3 standard deviations + [illegible] .sup.b Quantity of protein present in stock preparations of antigen before [illegible] the solid phase .sup.c Net absorbance values exceeding critical regions considered positive

Detailed Description Paragraph Table (2):
TABLE 2 Reactivity of serum samples from persons with Lyme disease, syphilis, or relapsing fever with whole cells or fractions of B. burgdorferi in ELISA No. of serum No. (%) positive.sup.a to B. burgdorferi Test samples whole Fractions groups tested [illegible]

[illegible]

disorders. .sup.d The sample reacted to whole cells and fractions B and C at 1:5120 but was depleted before being screened against fractions, E, D and F.

Detailed Description Paragraph Table (3):
TABLE 3 Reactivity of serum samples from persons with Lyme disease to whole- cell or fractions of B. burgdorferi in ELISA for IgG antibody Reciprocal IgG antibody titers.sup a Pa- whole Fractions tient.sup.b cell B C E D F ED 20,480 20,480 20,480 5,120 640 5,120 MP 10,240 1,280 N.sup.c N N 5,120 PD 10,240 20,480 1,280 2,560 320 1,280 RB 5,120 20,480 320 1,280 1,280 640 KZ 5,120 20,480 1,280 2,560 320 10,240 RR 5,120 10,240 2,560 640 2,560 20,480 BB 2,560 5,120 1,280 320 640 2,560 FM 1,280 10,240 5,120 2,56 N N FW 1,280 5,120 N 1,280 640 640 FM 640 2,560 5,120 2,560 N N JW 640 1,280 640 N N 640 JE 640 N N N 1,280 N .sup.a N = Negative (<1:160) .sup.b Persons had erythema migrans and one or more later manifestations of Lyme disease.

Detailed Description Paragraph Table (4):
Number of positive in ELISA Patient serum (n) OGP DGP 3-10 Normal individuals (4) 0 0 0 Rheumatoid factor (4) 0 0 0 Anti nuclear antibody (2) 0 0 0 Wasserman positive (3) 0 0 0 Borrelliosis (3) 3 3 3

Detailed Description Paragraph Table (5):
TABLE 4 Reactivity of Borrelia serum (dilution 1/200) in early and late stage with fractions of B. burgdarferi in ELISA Fractions B D E A.sub.405 A.sub.405 A.sub.405 Serum x .+- . SD % pos. n x .+-. SD % pos. n x .+-. SD % pos. n. Early 0.29 .+-. 0.18 55 10(18) 0.21 .+-. 0.16 20 3(15) 0.067 .+-. 0.031 18 2(11) stage Late 1.280 .+ . 0.38 100 10(10) 0.29 .+-. 0.19 63 7(11) 0.22 .+-. 0.093 11 1(9) stage

Detailed Description Paragraph Table (6):
TABLE 5 Specificity of B. burgdorferi fraction B measured as reactivity of various patient serum samples in ELISA No. of serum Cross reactivity Serum samples tested No. (%) Rheumatoid factor 49 0 (0) Anti-nuclear antibody 70 1 (1) Wassermann positive 10 0 (0) Syphilis 9 0 (0) Reactive arthritis 10 0 (0) Mononucleosis 13 1 (8)

Other Reference Publication (8):
Dunn, J. John et al. "Outer Surface Protein A (OspA) from the lyme Disease Spirochete, Borrelia burgdorferi: high level expression and purification of soluble recombinant form of OspA", Protein Expression and Purification, vol. 1, pp. 159-168 (1990).

Other Reference Publication (10):
Fraser, Claire M et al. "Genomic sequence of a Lyme disease spirochaete, Borrelia burgdorferi", Nature, vol. 390, Dec. 1997, pp. 580-586.

Other Reference Publication (20):
Ochs, Ridgely: "Vaccine takes aim at lyme disease, Newsday Jun. 16, 1997, and Results are in on 2nd Lyme Vaccine" Newsday Jun. 17, 1997.

CLAIMS:

1. A Lyme disease vaccine comprising a pharmaceutically acceptable carrier and a viral vector containing DNA encoding substantially pure OspA protein of Borrelia burgdorferi, or an immunogenic fragment thereof, whereby said viral vector expresses substantially pure OspA protein of Borrelia burgdorferi or an immunogenic fragment thereof, and does not express other Borrelia proteins.

2. The Lyme disease vaccine of claim 1 wherein said DNA encodes substantially pure OspA protein of Borrelia burgdorferi.

3. The Lyme disease vaccine of claim 1 wherein said DNA encodes an immunogenic fragment of said substantially pure OspA protein of Borrelia burgdorferi.

4. The Lyme disease vaccine of any one of claims 1 to 3 wherein said DNA is in [illegible] nucleotide sequence encoding a signal peptide for which there is an [illegible] and [illegible]

consisting of isoleucine and alanine.

11. The Lyme disease vaccine of claim 9 wherein said signal peptide has a C-terminal region containing an amino acid sequence L-I-x-C wherein x is a non-charged amino acid residue.

12. The Lyme disease vaccine of claim 11 wherein x is alanine.

13. The Lyme disease vaccine according to any one of claims 5 to 7 wherein said substantially pure OspA protein of Borrelia burgdorferi includes an amino acid sequence (I/L)-x-x-x-x-(I/L)-x-L-A-L-I-x-C wherein x is a non-charged amino acid residue and (I/L) denotes an amino acid residue selected from the group consisting of isoleucine and leucine.

14. The Lyme disease vaccine according to any one of claims 5 to 7 wherein said substantially pure OspA protein of Borrelia burgdorferi is a lipoprotein.

15. The Lyme disease vaccine of claim 14 wherein said substantially pure OspA protein of Borrelia burgdorferi has a fatty acylated cysteine as a first amino acid.

16. The Lyme disease vaccine according to any one of claims 5 to 7 wherein said substantially pure OspA protein of Borrelia burgdorferi contains an amino acid sequence selected from the group consisting of: Lys-Gly-Lys-Asn-Lys-Asp, Ser-Lys-Lys-Thr-Lys-Asp, and Lys-Ala-Asp-Lys-Ser-Lys.

17. The Lyme disease vaccine of claim 5 wherein said DNA has a nucleotide sequence encoding an amino acid sequence shown in FIG. 5 for the 31 kd substantially pure OspA protein of the New York strain B31 (ATCC 35210) of Borrelia burgdorferi.

18. The Lyme disease vaccine of claim 5 wherein said substantially pure OspA protein is the 31 kd OspA protein of the New York strain B31 (ATCC 35210) of Borrelia burgdorferi.

19. The Lyme disease vaccine according to any one of claims 17 or 18 wherein said substantially pure OspA protein of Borrelia burgdorferi is a lipoprotein.

20. A Lyme disease vaccine comprising a pharmaceutically acceptable carrier and a viral vector; said vector consisting essentially of DNA encoding substantially pure OspA protein of Borrelia burgdorferi, or an immunogenic fragment thereof, whereby said viral vector expresses substantially pure OspA protein of Borrelia burgdorferi or an immunogenic fragment thereof.

21. The Lyme disease vaccine of claim 20 wherein said DNA encodes substantially pure OspA protein of Borrelia burgdorferi.

22. The Lyme disease vaccine of claim 20 wherein said DNA encodes an immunogenic fragment of said substantially pure OspA protein of Borrelia burgdorferi.

23. The Lyme disease vaccine of any one of claims 21 to 22 wherein said DNA includes a nucleotide sequence encoding a signal peptide for which there is an amino acid recognition sequence.

24. The Lyme disease vaccine of claim 23 wherein said recognition sequence is L-x-x-C, wherein each x independently from the other represents a small neutral amino acid, and said substantially pure OspA protein of Borrelia burgdorferi is cleaved at this site in the expressing of said DNA.

25. The Lyme disease vaccine of claim 24 wherein said x is selected from the group consisting of isoleucine and alanine.

26. The Lyme disease vaccine of claim 25 wherein said signal peptide has a C-terminal

(I/L) denotes an amino acid residue selected from the group consisting of isoleucine and leucine.

29. The Lyme disease vaccine according to any one of claims 20 to 22 wherein said substantially pure OspA protein of Borrelia burgdorferi is a lipoprotein.

30. The Lyme disease vaccine of claim 29 wherein said substantially pure OspA protein of Borrelia burgdorferi has a fatty acylated cysteine as a first amino acid.

31. The Lyme disease vaccine according to any one of claims 20 to 22 wherein said substantially pure OspA protein of Borrelia burgdorferi contains an amino acid sequence selected from the group consisting of: Lys-Gly-Lys-Asn-Lys-Asp, Ser-Lys-Lys-Thr-Lys-Asp, and Lys-Ala Asp Lys-Ser-Lys.

32. The Lyme disease vaccine of claim 20 wherein said DNA has a nucleotide sequence encoding an amino acid sequence shown in FIG. 5 for the 31 kd substantially pure OspA protein of the New York strain B31 (ATCC 35210) of Borrelia burgdorferi.

33. The Lyme disease vaccine of claim 20 wherein said substantially pure OspA protein is the 31 kd OspA protein of the New York strain B31 (ATCC 35210) of Borrelia burgdorferi.

34. The Lyme disease vaccine according to any one of claims 32 or 33 wherein said substantially pure OspA protein of Borrelia burgdorferi is a lipoprotein.

55. A diagnostic agent for the diagnosis of Borrelia burgdorferi infection in humans or animals, the agent comprising an isolated DNA molecule homologous to an isolated DNA molecule as claimed in any one claims 1 or 2 or 3 or 3 encoding an immunological active component from Borrelia burgdorferi.

Generate Collection

L10: Entry 25 of 41 File: USPT Jun 20, 2000

DOCUMENT-IDENTIFIER: US 6077518 A
TITLE: Flagella-less borrelia

Abstract Text (1):
This invention relates to flagella-less strains of Borrelia and to novel methods for use of the microorganisms as vaccines and in diagnostic assays. Although a preferred embodiment of the invention is directed to Borrelia burgdorferi, the present invention encompasses flagella-less strains of other microorganisms belonging to the genus Borrelia. Accordingly, with the aid of the disclosure, flagella-less mutants of other Borrelia species, e.g., B. coriacei, which causes epidemic bovine abortion, B. anserina, which causes avian spirochetosis, and B. recurrentis and other Borrelia species causative of relapsing fever, such as Borrelia hermsii, Borrelia turicatae, Borrelia duttoni, Borrelia persica, and Borrelia hispanica, can be prepared and used in accordance with the present invention and are within the scope of the invention. Therefore, a preferred embodiment comprises a composition of matter comprising a substantially pure preparation of a strain of a flagella-less microorganism belonging to the genus Borrelia.

Brief Summary Text (2):
This invention relates to flagella-less strains of Borrelia and in particular, Borrelia burgdorferi, and to novel methods for use of the microorganisms as vaccines and in diagnostic assays, particularly for Lyme disease.

Brief Summary Text (4):
Lyme disease is a common tickborne infection of the northern hemisphere's temperate latitudes. The clinical features and epidemiology of Lyme disease have been well-characterized, and the etiologic agent, the spirochete Borrelia burgdorferi, has been isolated (reviewed by Steere, 1989). Borrelia burgdorferi enters the host's vascular system from the tick bite site and then is distributed to different organs and tissues, including the brain and joint synovium. In these different tissues the microorganism can persist for months to years. The properties of Borrelia burgdorferi that confer invasiveness in the human and other mammalian hosts have yet to be completely identified, although the flagellum has been implicated in pathogenicity.

Brief Summary Text (5):
Diagnosis of Lyme disease is complicated by the fact that the disease may mimic several other disorders, many of which are not infectious and, therefore, not ameliorated by antibiotics. A challenge for physicians is to identify cases of pauciarticular arthritis, radiculopathy, or extreme chronic fatigue as Lyme disease. If the clinical impression is confirmed by specific diagnostic assays, appropriate antimicrobial therapy may reverse long-standing pathological changes. Unfortunately, physicians are often frustrated in this process by the inadequacies of currently available diagnostic procedures.

Brief Summary Text (6):
Recovery of Borrelia burgdorferi from patients is possible and should be considered diagnostic. However, the medium is expensive to keep stocked, cultures require up to 4 weeks of incubation for routine detection of spirochetes, and the frequency of isolating bacteria from the blood of acutely ill patients is less than 5%. Consequently, [illegible] B. burgdorferi is only [illegible]

monoclonal and polyclonal antibodies has also been used successfully to show the presence of borreliae, but there is less experience with this technique than with the silver stains. (Park, et al., 1986).

Brief Summary Text (8):
Cases not meeting the strict clinical and epidemiologic criteria for diagnosis have also been identified as Lyme disease by using a serologic test, usually an enzyme-linked immunosorbent assay (ELISA) or in-direct immunofluorescence assay (IFA). Although many public and private laboratories now offer either ELISA or IFA, the procedures for these assays have not yet been standardized. The antigen preparations and the "cut-off" values for a positive test vary among laboratories. Significant interlaboratory variations in test results and in interpretations of the same set of sera have been reported (Hedberg, et al., 1987).

Brief Summary Text (9):
Many present immunoassays use whole spirochetes or a crude sonicate of the cells. However, those assays suffer from complications resulting from cross-reactions with other spirochetes, especially Treponema palladium and the relapsing fever Borrelia species (Magnarelli and Anderson, 1988), and borderline or low-level positive titers in some patients with other rheumatologic or neurologic disorders. Because of increasing professional and lay awareness of Lyme disease, serologic testing is often requested to "rule out" the diagnosis. In this situation, the ratio of persons with false-positive reactions compared with those who have actual Borrelia burgdorferi infections will predictably rise. Thus, "seropositive" patients with disorders other than Lyme disease may be subjected to long and possibly hazardous courses of oral or parenteral antibiotics. A more specific diagnostic assay for B. burgdorferi is needed.

Brief Summary Text (10):
Although several investigators have suggested use of an immunoassay using a purified flagella protein antigen, there are problems with this approach. For one thing, it has recently been shown that a monoclonal antibody directed against the major flagella protein of Borrelia burgdorferi also recognizes human tissue, including myelin and Schwann cells from the peripheral nervous system (Sigal, et al., 1988; Aberer, et al., 1989). Autoantibodies against neural antigens have been observed in the serum of patients with Lyme disease. These findings suggest that autoreactive antibodies may complicate interpretation of immunoassays that use purified flagellar antigen or whole cell sonicates containing flagella antigen. Another problem with the use of preparations containing the flagellum by itself or in combination with other components is that there may be false positive reactions as a consequence of antigenic similarities between the flagella of borreliae and the flagella of other bacteria.

Brief Summary Text (11):
The discovery that flagellar antigens may induce formation of antibodies reactive with human neural tissue provides an additional problem with respect to development of a vaccine against Lyme Disease. International Patent Application No. WO 90/04411, published May 3, 1990 describes a method for preparing fractions of Borrelia burgdorferi in which the flagellar components are at least partially depleted. However, that method is somewhat time consuming and labor intensive. Inactivated whole cell

Brief Summary Text (12):
Borrelia burgdorferi vaccines, such as those described in U.S. Pat. No. 4,721,617 and by Johnson, et al., 1986, comprise a relatively high proportion of the flagellar antigen and may thus induce an undesirable auto immune response. In addition, because flagellated Borrelia is virulent, the cells must be killed prior to administration, thus reducing immunogenicity. Because the flagellum is an important virulence factor of the organism, development of a flagellar-less strain could provide an ideal approach for development of an attenuated vaccine.

Brief Summary Text (13):
Many of the problems set forth above have been overcome by the present invention, which provides a novel flagella less strain of Borrelia burgdorferi suitable for use as

be administered as a live vaccine. Moreover, the organism may be used in immunoassay, alone or in conjunction with a flagellated strain, without the complications of the potential contribution of auto-antibodies or antibodies cross-reactive with borrelial flagella resulting from immunogenic exposure to non-borrelial flagella.

Brief Summary Text (16):
Although a preferred embodiment of the invention is directed to Borrelia burgdorferi, the present invention encompasses any flagella-less strain of a microorganism belonging to the genus Borrelia. Accordingly, with the aid of the present disclosure, flagella-less mutants of other Borrelia species, e.g., B. coriacei, which causes epidemic bovine abortion, B. anserina, which causes avian spirochetosis, and B. recurrentis and other Borrelia species causative of relapsing fever, such as Borrelia hermsii, Borrelia turicatae, Borrelia duttoni, Borrelia persica, and Borrelia hispanica, can be prepared and used in accordance with the present invention and are within the scope of the invention. Thus, the invention includes a culture of the flagella-less borreliae, a composition of matter comprising a substantially pure preparation of a flagella-less strain of such a microorganism, and a composition of matter comprising a purified preparation of antigens derived from a culture of a flagella-less strain of such a microorganism.

Brief Summary Text (17):
Also included is an immunoassay procedure for detection of Borreliosis, i.e., infection with borreliae, comprising obtaining a biological sample, such as a sample of a bodily fluid, such as blood, serum, plasma, urine, or synovial or cerebrospinal fluid, from an individual to be tested, contacting the sample with an antigenic preparation derived from a culture of a selected flagella-less strain of borreliae under conditions suitable to allow binding between the antigens in the preparation and borreliae-reactive antibodies in the sample and detecting the binding.

Brief Summary Text (18):
So called "agglutination" assays may be used in accordance with the present invention. In one example, a "latex agglutination" assay, the antigenic preparation is adsorbed or chemically coupled to a particle, such as a latex bead, and particles bearing the antigen are agglutinated under conditions which allow crosslinking of antigen molecules on discrete particles by antibody-antigen complex formation. Alternatively, the antigenic preparations can be used in a so called "microagglutination" or "flocculation" assay, where clumps of antigen-antibody complexes are observed directly.

Brief Summary Text (19):
In another embodiment, the immunoassay may comprise what is known to those of skill in the art as a competitive immunoassay, in such an assay binding is detected by adding a preparation of labeled antibodies reactive with a selected flagella-less Borrelia strain to the contacted sample and measuring binding of the labeled antibody to the antigenic preparation. Because antibodies in the sample will compete with the labeled antibodies for antigenic epitopes in the antigen preparation, binding of the labeled antibody will be inversely proportional to the concentration of antibody in the sample.

Brief Summary Text (20):
Alternatively, the immunoassay procedure may be an immunoassay wherein the binding is detecting by adding to the contacted sample a preparation of labeled antibodies that are capable of binding to the antibodies in the sample (e.g., anti immunoglobulin antibodies) under conditions suitable to allow binding between the labeled antibodies and the antibodies in the samples and measuring the amount of the labeled antibody bound to the antigen-bound antibodies.

Brief Summary Text (21):
Any of a number of different detectable labels known to those of skill in the art for use in immunoassay may be used in these procedures, including, for example, radioactive labels, fluorescent labels, enzymatic labels such as peroxidase capable of cleaving a

Brief Summary Text (22):
The invention also includes procedures for detecting antibodies capable of binding to non-flagellar antigens of microorganisms of a selected Borrelia species, preferably Borrelia burgdorferi. Those procedure comprises obtaining a sample to be tested for the antibodies, contacting the sample with an antigenic preparation, derived from a culture of a flagella-less strain of the selected Borrelia species, under conditions suitable to allow binding between the antigens in the preparation and the antibodies, and detecting the binding. In a preferred embodiment, the immunoassay will comprise a solid-phase immunoassay procedure for detecting antibodies capable of binding to non-flagellar antigens of a selected Borrelia species. That assay comprises immobilizing an antigenic preparation derived from a culture of a flagella-less strain of the Borrelia species on a solid matrix, contacting the immobilized preparation with a sample to be tested for the presence of the antibodies under conditions suitable to allow binding between the antigens in the preparation and the antibodies, separating antibodies bound to the immobilized antigens from the remainder of the sample, and detecting the antigen-bound antibodies. Also included is an additional solid-phase immunoassay for detecting antibodies capable of binding to non-flagellar antigens of a selected Borrelia species comprising obtaining a sample from an individual to be tested, immobilizing antibodies present in the sample on a solid matrix, separating the immobilized antibodies from the sample, contacting the immobilized antibody with an antigenic preparation derived from a flagella-less strain of the selected Borrelia species under conditions suitable to allow binding between the antigens in the preparation and the antibodies, and detecting the antigen bound antibodies. With this method, the antibodies are detected by contacting the antigen-bound antibody with a detectably labeled antibody capable of specifically binding to the antigen-bound antibody under conditions suitable to allow the binding to occur.

Brief Summary Text (24):
The invention also includes a number of kits for immunoassay. Such kits may comprise, for example, a carrier compartmentalized to contain one or more containers, and a first container containing an antigenic preparation derived from a flagella-less strain of a selected Borrelia species. In one embodiment, the antigenic preparation may be provided immobilized on a solid phase, such as a microtiter well or latex bead. The kit may further comprise a second container comprising a preparation of antibodies reactive with the antigens in the antigenic preparation, and/or a third container containing a detection reagent.

Brief Summary Text (25)
The invention includes vaccines for Borrelliosis and vaccination procedures. Vaccines according to the present invention may comprise, for example, an antigenic component derived from a culture of a flagella-less Borrelia strain and a pharmaceutically acceptable carrier. As with other aspects of the invention, the vaccine can comprise any of a number of selected Borrelia species, including but not limited to B. coriacei, for prevention of epidemic bovine abortion; B. recurrentis, B. hermsii, B. turicatae, B. duttoni, B. persica, and B. hispanica, for prevention of relapsing fever; and B. anserina for prevention of avian spirochetosis. Preferably, however, the vaccine will be preventative of Lyme disease in which case it will include an antigenic component derived from a flagella-less strain of B. burgdorferi.

Brief Summary Text (26):
The invention also includes a method for inducing an immune response in a mammal or bird to a microorganism belonging to the genus Borrelia comprising administering an immunogenic dose of the vaccine to the mammal or bird. As those of skill in the art will appreciate, a number of mammals are infected with or are carriers of Borrelia pathogens and thus the invention is not limited by a particular mammal to be injected with the vaccine derived from a particular Borrelia species. However, preferred combinations are those most likely to elicit control or prevention of a commercially significant pathogen. Consequently, vaccines comprising antigens from B. burgdorferi will usually be administered to the primary victims or carriers of Lyme disease such as humans, dogs, horses, equids, cattle, victims, deer and rodents, particularly mice

vaccines possess the additional advantage of facilitating diagnosis of Borreliosis in individuals, mammals, and birds who have been administered the vaccine. As those of skill in the art will recognize, few if any vaccines are one hundred percent efficacious and vaccine failures do occur. Furthermore, when the disease vaccinated against is a disease which, like Lyme disease, elicits symptoms that can be attributed to a number of other pathologic conditions, specific immunodiagnostic assays may be complicated by antibodies elicited against the vaccine. In contrast, when the flagella-less microorganisms of the present invention are used for immunization, one can simply assay an individual exhibiting symptoms characteristic of a selected borrelial pathogenesis, such as Lyme disease, for antibodies to the flagellar-antigen. In such individuals, the absence of such antibodies will usually weigh against a diagnosis of Borreliosis, and their presence will be suggestive of a vaccine failure.

Drawing Description Text (6):
FIG. 5: Comparison of W, M, and R cells of Borrelia burgdorferi strain HB19 in an ELISA with Lyme disease patient and control sera. The x and y axes are the absorbance values from the assays. Each point denotes the result of each serum in the pairwise comparison. When absorbance results with sera were .gtoreq.1.500 with either of the antigens in the comparison, a single point is shown.

Detailed Description Text (3):
The flagella-less Borrelia strains of the present invention may be advantageously used in immunoassay procedures or as vaccine components.

Detailed Description Text (4):
Suitable immunoassays for use with the flagella-less Borrelia strains of the invention include assays employing a number of principles well known to those of skill in the art, including those described by Nisonoff, Introduction to Molecular Immunology, 2nd Ed., Sinauer Associates, Inc., Sunderland, Mass. (1984) and in U.S. Pat. No. 4,376,110, both incorporated herein by reference.

Detailed Description Text (5):
Generally, for detection of antibody in biologic samples, such as blood, plasma, serum, cerebrospinal fluid, synovial fluid, urine and the like, the flagella-less borreliae as antigen, or an antigenic composition prepared therefrom, is preferably adsorbed, or otherwise attached, to an appropriate adsorption matrix (for example, the inside surface of a microtiter dish or well) and a sample of a suspected antibody-containing composition is contacted therewith to cause formation of an immunocomplex between antigens in the composition and any antibodies in the sample that bind to those antigens. The matrix is then washed to remove non-specifically bound material and the immunocomplexes are detected, typically through the use of an appropriate labeled ligand.

Detailed Description Text (6):
Antigen compositions comprising flagella-less borreliae may also be incorporated into diagnostic kits useful in performing assays of the type described above. A number of kits might be utilized in the practice of the present invention, for example, a kit comprising a carrier compartmentalized to contain at least one, at least two, or at least three or more containers.

Detailed Description Text (7):
A first container may include a composition comprising an antigen preparation of the flagella-less borreliae, which may include whole cell preparations or lysates of flagella-less microorganisms or preparations including partially or substantially purified antigenic components derived therefrom, and in particular, cell surface protein antigens. The kits may also include antibody compositions having specificity for one or more Borrelia antigens. Both antibody and antigen preparations should preferably be provided in a suitable titrated form, with antigen concentrations and/or antibody titers given for easy reference in quantitative applications, although the antigenic preparation may also be provided immobilized on a solid matrix.

Detailed Description Text (8):
[illegible]

Immunodetection reagents and processes suitable for application in connection with the novel compositions of the present invention are generally well known in the art.

Detailed Description Text (9):
The flagella-less borreliae of the invention may also be effectively used as vaccines to prevent Borreliosis, and Lyme disease in particular. In general, immunogenic compositions suitable for administration as vaccines could be formulated to include the flagella-less borreliae, whole cell lysates thereof, or purified antigenic preparations derived from the flagella-less borreliae. Typically, such vaccines are prepared as injectables, either as liquid solutions or suspensions, although solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The preparations may also be emulsified. The reactive immunogenic ingredient is often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the vaccine could contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, or adjuvants which enhance the effectiveness of the vaccine.

Detailed Description Text (10):
The antigens can be formulated into the vaccine as neutral or salt forms and administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective and immunogenic. The vaccines may be conventionally administered parenterally, by injection, for example, either subcutaneously or intramuscularly. Where a live vaccine is used, preferred modes of administration are subcutaneous and intradermal injection. Additional formulations which are suitable for other modes of administration may include oral or intranasal formulations. The quantity to be administered will depend on the subject to be treated, capacity of the immune system to synthesize antibodies, and the degree of protection desired. Precise amounts of active ingredient required to be administered will depend on the judgment of the practitioner and may be peculiar to each individual. However, suitable dosage ranges may be on the order of 0.01 ug to 10 mg, and more preferably 1 to 100 ug active ingredient per kilogram of body weight. Suitable regimes for initial administration and booster shots will also be variable, but may be typified by an initial administration followed by subsequent inoculations or other administrations.

Detailed Description Text (11):
In many instances, it may be desirable to have multiple administrations of the vaccine, at from two to twelve week intervals, more usually from three to five week intervals. Periodic boosters at intervals of 1-5 years, usually three years, will be desirable to maintain protective levels of the antibodies. The course of the immunization may be followed by assays for antibodies for the antigens as described above.

Detailed Description Text (32):
Even with long exposures, full length or truncated flagellin protein was not detectable. Western blots with polyclonal rabbit antisera to Borrelia burgdorferi and Lyme disease patient sera confirmed that M cells appeared to differ from W and R cells only in the absence of a major antigen of approximately 37 kDa (data not shown).

Detailed Description Text (33):
Although the absence of flagellin within the cells of the non motile mutant was probably the result of failure of expression of the flagellin protein itself, another possible explanation of the findings is that flagellin was produced by the cells but it was not anchored to the basal proteins, and hence lost to the medium. This seemed unlikely, because even transiently associated flagellin should have been detectable in the cells by Western blot. Nevertheless, this alternative explanation was tested by intrinsically labeling the different isolates with .sup.35 S-methionine during growth and then examining the supernatant for evidence of immunoreactive flagellin polypeptides or peptide fragments by immunoprecipitation. The study did not detect the greater presence of flagellin cross reactive antigens in medium containing the flagella-less

detectably lower when the flagella-less mutant was used as an antigen than when its flagella-bearing counterparts were used. Sera from patients and controls were examined for their reactivities against whole cells of W, M, and R when equivalent amounts of total cellular protein were used as antigens. Sera from 17 adult patients with Lyme disease of 6 or more weeks duration from Connecticut, Wisconsin, and Lithuania, areas with high incidence of Lyme disease, were used. Sera from 18 healthy adult residents of Rocky Mountain states, a region with a low incidence of Lyme disease, were used as controls.

Detailed Description Text (45):
The enzyme-linked immunoabsorbent assay (ELISA) using whole cells of Borrelia burgdorferi was a modification of the method of Magnarelli et al. (Magnarelli and Anderson, 1988). Harvested borreliae were suspended in PBS/Mg and an estimate of total cellular protein in the suspension was made using the Bradford assay (Bio-Rad Laboratories, Richmond, Calif.).

Detailed Description Text (46):
The suspension was diluted 1:1000 in a volume of carbonate buffer (Magnarelli and Anderson, 1988) that gave a protein concentration of 1.4 mg/ml; 50 .mu.l of the diluted cell suspension was added to each well of a flat-bottomed, polystyrene microtiter plate (Corning). After incubation of the plates for 18 hrs at 37.degree. C., 200 ul of 1% (w/v) dried nonfat milk in PBS was added to each dry well. Plates were incubated for 1 hour at 37.degree. C. and washed 4 times with 200 ul of PBS. The plates were incubated for 1 hour at 37.degree. C., and then washed with PBS. Bound IgG antibody was assayed with horseradish peroxidase-conjugated, anti-human IgG (gamma-chain specific) goat antisera (Cal-Biochem, San Diego, Calif.) in 1% nonfat milk/PBS buffer. After incubation for 1 hr. at 37.degree. C., the plates were washed 4 times with 200 ul of PBS. The substrate for the peroxidase reaction was O-phenylenediamine dihydrochloride, and absorbance values at 490 mM were recorded on a Dynatech ELISA reader (Model #580). The maximum absorbance value read was 1.5000.

Detailed Description Text (47):
The mean absorbance values (.+-.standard error) for the 17 patient sera were 1.09 (.+-.0.09) for W, 1.31 (.+-.0.08) for M, and 1.15 (.+-.0.09) for R cells. Analogous determinations for 18 control sera were 0.19 (.+-.0.03), 0.19 (.+-.0.03), and 0.20 (.+-.0.03), respectively. FIG. 5 shows the pairwise comparisons for W, M, and R cells when patient and control sera were used in the ELISA. There was good correlation between W and R cells for patient sera. Furthermore, when control sera were used the 3 isolates gave comparable results. Surprisingly, the flagella-less M cells had an equal or higher absorbance reading for each patient serum when compared with W and R cells. Many of the values with M cells in the ELISA were .gtoreq.1.50, the absorbance reading maximum. When sera were diluted 1:1000 instead of 1:500 consistently higher absorbance values with M cells as compared with W and R cells was observed (data not shown).

Detailed Description Text (57):
From these studies we concluded that flagella less strains could be isolated from Borrelia burgdorferi cultures and maintained in culture. Furthermore, the absence of flagella does not reduce the efficacy of a Borrelia burgdorferi for serologic testing for antibodies to Borrelia burgdorferi in patients with Lyme borreliosis. Indeed, the use of a flagellaless microorganism in a serologic assay is likely to improve the sensitivity and specificity of the assay. Although applicants are not bound by theory, the possible reasons for this are two fold: (a) When flagella are not present, the relative amounts of other proteins in the suspension or a protein concentration basis increases. One or more of these other proteins or non proteins may be more important than flagella for diagnostic purposes. (b) Flagella are likely a major source of false-positive reactions, because of the cross-reactivities between the flagella of Borrelia burgdorferi and those of other bacteria. When flagella are not present, the specificity of the assay may improve. The flagella-less isolate could be used in place of borrelia with the wild type phenotype in immunofluorescence, ELISA base, and Western blot assays.

In additional studies, rats were immunized intramuscularly with 20 .mu.g total protein of borreliae in complete Freund's adjuvant. After 6 weeks, the rats were bled and their serum was analyzed by ELISA, essentially as described above. The results of this experiment shown in Table 3, indicated that immunization with M cells induced a significant immune response against W cells.

Detailed Description Text (69):
As shown, serum from immunized rats, even at a dilution of 1:3,000 was capable of neutralizing B. burgdorferi. Thus, when used to immunize rats, flagella-less (M) cells were as effective as flagella-bearing (W) cells in eliciting antibodies to B. burgdorferi as determined by ELISA and by growth inhibition assay.

Detailed Description Text (72):
For example, numerous methods for immunoassay may be used. In addition, variations in vaccine preparation may be employed. It is apparent that the invention may also be utilized, with suitable modifications within the state of the art. It is the Applicants intention in the following claims to cover all such equivalent modifications and variations which fall within the true spirit, and scope of the invention.

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Detailed Description Text (78):
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Detailed Description Text (81):
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Detailed Description Text (82):
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Detailed Description Text (86):
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Detailed Description Text (93):
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Detailed Description Text (95):
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Detailed Description Text (96):
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Detailed Description Text (105):
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Detailed Description Text (115):
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Detailed Description Text (124):
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Detailed Description Text (125):
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Detailed Description Text (129):
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Detailed Description Text (130):
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Detailed Description Text (132):
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Detailed Description Paragraph Table (3):

TABLE 3 ELISA Test With Serum Diluted 1:1,000

Absorbance Rat #	Immunogen	ELISA antigen	Value
1	W cells	W cells	0.713
	M cells	0.727	
2	W cells	W cells	0.592
	M cells	0.629	
3	M cells	W cells	0.555
	M cells	0.449	
4	M cells	W cells	0.589
	M cells	1.082	
5	Adjuvant alone	W cells	0.010
	M cells	0.014	
6	Adjuvant alone	W cells	0.015
	M cells	0.013	

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25. Document ID: US 6077515 A

L10: Entry 25 of 41 File: USPT Jun. 20, 2000

US-PAT-NO: 6077515
DOCUMENT-IDENTIFIER: US 6077515 A

TITLE: Flagella-less borrelia

DATE-ISSUED: June 20, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Barbour; Alan G.	San Antonio	TX		
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US-CL-CURRENT: 424/234.1; 424/184.1, 424/185.1, 424/282.1, 424/93.1, 424/93.4, 435/243, 435/245, 435/252.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

26. Document ID: US 6068842 A

L10: Entry 26 of 41 File: USPT May 30, 2000

US-PAT-NO: 6068842
DOCUMENT-IDENTIFIER: US 6068842 A

TITLE: 66 kDa antigen from Borrelia

DATE-ISSUED: May 30, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
[illegible]	[illegible]			[illegible]
Barbour; Alan George	San Antonio	TX		

US-CL-CURRENT: 424/184.1; 424/271.1, 424/234.1, 435/69.1, 435/69.3, 530/350

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

delivery

DATE-ISSUED: May 9, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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US-CL-CURRENT: 424/450; 436/829

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claim | Draw Desc | Image

28. Document ID: US 6054296 A

L10: Entry 28 of 41 File: USPT Apr 25, 2000

US-PAT-NO: 6054296
DOCUMENT-IDENTIFIER: US 6054296 A

TITLE: 66 kDa antigen from Borrelia

DATE-ISSUED: April 25, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bergstrom; Sven	Umea			SE
Barbour; Alan George	San Antonio	TX		

US-CL-CURRENT: 435/69.3, 424/184.1, 424/234.1, 424/262.1, 435/320.1, 435/69.1, 536/23.1, 536/23.4, 536/23.7

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claim | Draw Desc | Image

29. Document ID: US 6040157 A

L10: Entry 29 of 41 File: USPT Mar 21, 2000

US-PAT-NO: 6040157
DOCUMENT-IDENTIFIER: US 6040157 A

TITLE: Vascular endothelial growth factor 2

DATE-ISSUED: March 21, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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Cao; Liang	South Harrow			HK

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claim | Draw Desc | Image

30. Document ID: US 6004534 A

L10: Entry 30 of 41 File: USPT Dec 21, 1999

US-PAT-NO: 6004534
DOCUMENT-IDENTIFIER: US 6004534 A

TITLE: Targeted polymerized liposomes for improved drug delivery

DATE-ISSUED: December 21, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Langer; Robert S.	Newton	MA		
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US-CL-CURRENT: 424/9.321; 264/4.1, 264/4.3, 424/184.1, 424/278.1, 424/450, 424/812, 424/9.4, 424/9.51, 424/9.52, 428/402.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | DWIC | Draw Desc | Image

31. Document ID: US 5977339 A

L10: Entry 31 of 41 File: USPT Nov 2, 1999

US-PAT-NO: 5977339
DOCUMENT-IDENTIFIER: US 5977339 A

TITLE: Methods and compositions for diagnosing lyme disease

DATE-ISSUED: November 2, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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US-CL-CURRENT: 536/24.32; 424/184.1, 435/320.1, 435/69.3, 435/7.2, 536/23.1, 536/23.7, 536/24.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | DWIC | Draw Desc | Image

32. Document ID: US 5965702 A

L10: Entry 32 of 41 File: USPT Oct 12, 1999

US-PAT-NO: 5965702
DOCUMENT-IDENTIFIER: US 5965702 A

TITLE: Borrelia burgdorferi antigens and uses thereof

DATE-ISSUED: October 12, 1999

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments Link | Draw Desc | Image

33. Document ID: US 5837263 A

L10: Entry 33 of 41 File: USPT Nov 17, 1998

US-PAT-NO: 5837263
DOCUMENT-IDENTIFIER: US 5837263 A

TITLE: Leptospira membrane proteins

DATE-ISSUED: November 17, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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US-CL-CURRENT: 424/234.1; 424/190.1, 435/69.1, 435/69.3, 435/71.1, 530/359

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments Link | Draw Desc | Image

34. Document ID: US 5643751 A

L10: Entry 34 of 41 File: USPT Jul 1, 1997

US-PAT-NO: 5643751
DOCUMENT-IDENTIFIER: US 5643751 A

TITLE: Borrelia burgdorferi antigens and uses thereof

DATE-ISSUED: July 1, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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Hunt; Jeffrey C.	Lindenhurst	IL		

US-CL-CURRENT: [illegible]

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments Link | Draw Desc | Image

35. Document ID: US 5643733 A

L10: Entry 35 of 41 File: USPT Jul 1, 1997

US-PAT-NO: [illegible]

NAME	CITY	STATE	ZIP CODE	COUNTRY
Robinson; John M.	Gurnee	IL		
Pilot-Matias; Tami J.	Libertyville	IL		
Hunt; Jeffrey C.	Lindenhurst	IL		

US-CL-CURRENT: 435/7.1; 435/7.2, 435/7.3, 435/7.32, 436/518

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Draw Desc | Image

36. Document ID: US 5620862 A

L10: Entry 36 of 41 File: USPT Apr 15, 1997

US-PAT-NO: 5620862
DOCUMENT-IDENTIFIER: US 5620862 A

TITLE: Methods for diagnosing early Lyme disease

DATE-ISSUED: April 15, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Padula; Steven J.	Simsbury	CT		

US-CL-CURRENT: 435/7.32; 435/7.92, 435/975, 436/513

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Draw Desc | Image

37. Document ID: US 5585102 A

L10: Entry 37 of 41 File: USPT Dec 17, 1996

US-PAT-NO: 5585102
DOCUMENT-IDENTIFIER: US 5585102 A

TITLE: Flagella-less borrelia

DATE-ISSUED: December 17, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Barbour; Alan G.	San Antonio	TX		
Bundoc; Virgilio S.	Newbury Park	CA		
Sadziene; Adriadna	San Antonio	TX		

US-CL-CURRENT: 424/234.1; 435/243, 435/245, 435/29, 435/40.5, 435/7.1, 435/7.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Draw Desc | Image

TITLE: T cell antigen receptor V region proteins and methods of preparation thereof

DATE-ISSUED: September 3, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Makrides; Savvas C.	Bedford	MA		
Kung; Patrick C.	Brookline	MA		

US-CL-CURRENT: 435/69.1; 435/252.3, 435/320.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | Draw Desc | Image

39. Document ID: US 5436000 A

L10: Entry 39 of 41 File: USPT Jul 25, 1995

US-PAT-NO: 5436000
DOCUMENT-IDENTIFIER: US 5436000 A

TITLE: Flagella-less borrelia

DATE-ISSUED: July 25, 1995

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Barbour; Alan G.	San Antonio	TX		
Bundoc; Virgilio	San Antonio	TX		

US-CL-CURRENT: 424/93.2; 424/234.1, 435/243, 435/245, 435/7.32

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | Draw Desc | Image

40. Document ID: US 5324630 A

L10: Entry 40 of 41 File: USPT Jun 28, 1994

US-PAT-NO: 5324630
DOCUMENT-IDENTIFIER: US 5324630 A

TITLE: Methods and compositions for diagnosing Lyme disease

DATE-ISSUED: June 28, 1994

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
LeFebvre; Rance B.	Davis	CA		
Perng; Guey-Chuen	San Gabriel	CA		

US-CL-CURRENT: 435/7.1; 435/7.21

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | Draw Desc | Image

41. Document ID: US 4888276 A

US-PAT-NO: 4888276
DOCUMENT-IDENTIFIER: US 4888276 A

TITLE: Method and composition for the diagnosis of Lyme disease

DATE-ISSUED: December 19, 1989

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Shelburne; Charles E.	Brooklyn Park	MN		

US-CL-CURRENT: 435/7.32; 436/548, 530/388.4

Term	Documents
IMMUNOASSAY.DWPI,EPAB,JPAB,USPT,PGPB.	25835
IMMUNOASSAYS.DWPI,EPAB,JPAB,USPT,PGPB.	15651
(9 AND IMMUNOASSAY).USPT,PGPB,JPAB,EPAB,DWPI.	41
(L9 AND IMMUNOASSAY).USPT,PGPB,JPAB,EPAB,DWPI.	41

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L11: Entry 4 of 4 File: USPT Jul 25, 1995

DOCUMENT-IDENTIFIER: US 5436000 A
TITLE: Flagella-less borrelia

Abstract Text (1):
This invention relates to flagella-less strains of Borrelia and to novel methods for use of the microorganisms as vaccines and in diagnostic assays. Although a preferred embodiment of the invention is directed to Borrelia burgdorferi, the present invention encompasses flagella-less strains of other microorganisms belonging to the genus Borrelia. Accordingly, with the aid of the disclosure, flagella-less mutants of other Borrelia species, e.g., B. coriacei, which causes epidemic bovine abortion, B. anserina, which causes avian spirochetosis, and B. recurrentis and other Borrelia species causative of relapsing fever, such as Borrelia hermsii, Borrelia turicatae, Borrelia duttoni, Borrelia persica, and Borrelia hispanica, can be prepared and used in accordance with the present invention and are within the scope of the invention. Therefore, a preferred embodiment comprises a composition of matter comprising a substantially pure preparation of a strain of a flagella-less microorganism belonging to the genus Borrelia.

Brief Summary Text (2):
This invention relates to flagella-less strains of Borrelia and in particular, Borrelia burgdorferi, and to novel methods for use of the microorganisms as vaccines and in diagnostic assays, particularly for Lyme disease.

Brief Summary Text (4):
Lyme disease is a common tickborne infection of the northern hemisphere's temperate latitudes. The clinical features and epidemiology of Lyme disease have been well-characterized, and the etiologic agent, the spirochete Borrelia burgdorferi, has been isolated (reviewed by Steere, 1989). Borrelia burgdorferi enters the host's vascular system from the tick bite site and then is distributed to different organs and tissues, including the brain and joint synovium. In these different tissues the microorganism can persist for months to years. The properties of Borrelia burgdorferi that confer invasiveness in the human and other mammalian hosts have yet to be completely identified, although the flagellum has been implicated in pathogenicity.

Brief Summary Text (5):
Diagnosis of Lyme disease is complicated by the fact that the disease may mimic several other disorders, many of which are not infectious and, therefore, not ameliorated by antibiotics. A challenge for physicians is to identify cases of peripheral arthritis, radiculopathy, or extreme chronic fatigue as Lyme disease. If the clinical impression is confirmed by specific diagnostic assays, appropriate antimicrobial therapy may reverse long-standing pathologic changes. Unfortunately, physicians are often frustrated in this process by the inadequacies of currently available diagnostic procedures.

Brief Summary Text (6):
Recovery of Borrelia burgdorferi from patients is possible and should be considered diagnostic. However, the culture is [illegible]

stain reveals spirochetes in one-half or more of skin biopsies obtained from the outer portion of lesions (Duray, 1987; Berger, et al., 1983). The microorganisms are comparatively sparse, however, and can be confused with normal skin structures by inexperienced laboratory personnel. Immunohistologic examination of tissue with monoclonal and polyclonal antibodies has also been used successfully to show the presence of borreliae, but there is less experience with this technique than with the silver stains. (Fark, et al., 1986).

Brief Summary Text (8):
Cases not meeting the strict clinical and epidemiologic criteria for diagnosis have also been identified as Lyme disease by using a serologic test, usually an enzyme-linked immunosorbent assay (ELISA) or in-direct immunofluorescence assay (IFA). Although many public and private laboratories now offer either ELISA or IFA, the procedures for these assays have not yet been standardized. The antigen preparations and the "cut-off" values for a positive test vary among laboratories. Significant interlaboratory variations in test results and in interpretations of the same set of sera have been reported (Hedberg, et al., 1987).

Brief Summary Text (9):
Many present immunoassays use whole spirochetes or a crude sonicate of the cells. However, those assays suffer from complications resulting from cross-reactions with other spirochetes, especially Treponema palladium and the relapsing fever Borrelia species (Magnarelli and Anderson, 1988), and borderline or low-level positive titers in some patients with other rheumatologic or neurologic disorders. Because of increasing professional and lay awareness of Lyme disease, serologic testing is often requested to "rule out" the diagnosis. In this situation, the ratio of persons with false-positive reactions compared with those who have actual Borrelia burgdorferi infections will predictably rise. Thus, "seropositive" patients with disorders other than Lyme disease may be subjected to long and possibly hazardous courses of oral or parenteral antibiotics. A more specific diagnostic assay for B. burgdorferi is needed.

Brief Summary Text (10):
Although several investigators have suggested use of an immunoassay using a purified flagella protein antigen, there are problems with this approach. For one thing, it has recently been shown that a monoclonal antibody directed against the major flagella protein of Borrelia burgdorferi also recognizes human tissue, including myelin and Schwann cells from the peripheral nervous system (Sigal, et al., 1988; Aberer, et al., 1989). Autoantibodies against neural antigens have been observed in the serum of patients with Lyme disease. These findings suggest that autoreactive antibodies may complicate interpretation of immunoassays that use purified flagellar antigen or whole cell sonicates containing flagella antigen. Another problem with the use of preparations containing the flagellum by itself or in combination with other components is that there may be false positive reactions as a consequence of antigenic similarities between the flagella of borreliae and the flagella of other bacteria.

Brief Summary Text (11):
The discovery that flagellar antigens may induce formation of antibodies reactive with human neural tissue provides an additional problem with respect to development of a vaccine against Lyme Disease. International Patent Application No. WO 9 4411, published May 5, 19 describes a method for preparing fractions of Borrelia burgdorferi in which the flagellar components are at least partially depleted. However, that method is somewhat time consuming and labor intensive. Inactivated whole cell Borrelia burgdorferi vaccines, such as those described in U.S. Pat. No. 4,721,617 and by Johnson, et al., 1986, comprise a relatively high proportion of the flagellar antigen and may thus induce an undesirable auto immune response. In addition, because flagellated Borrelia is virulent, the cells must be killed prior to administration, thus reducing immunogenicity. Because the flagellum is an important virulence factor of the organism, development of a flagellar-less strain could provide an ideal approach for development of an attenuated vaccine.

Brief Summary Text (14):

virulence of the organism, the vaccine of the present invention is likely to be safer than whole cell Borrelia burgdorferi preparations described in the past, and thus may be administered as a live vaccine. Moreover, the organism may be used in immunoassay, alone or in conjunction with a flagellated strain, without the complications of the potential contribution of auto-antibodies or antibodies cross-reactive with borrelial flagella resulting from immunigenic exposure to non-borrelial flagella.

Brief Summary Text (15):
Although a preferred embodiment of the invention is directed to Borrelia burgdorferi, the present invention encompasses any flagella-less strain of a microorganism belonging to the genus Borrelia. Accordingly, with the aid of the present disclosure, flagella-less mutants of other Borrelia species, e.g., B. coriaceI, which causes epidemic bovine abortion, B. anserina, which causes avian spirochetosis, and B. recurrentis and other Borrelia species causative of relapsing fever, such as Borrelia hermsii, Borrelia turicatae, Borrelia duttoni, Borrelia persica, and Borrelia hispanica, can be prepared and used in accordance with the present invention and are within the scope of the invention. Thus, the invention includes a culture of the flagella-less borreliae, a composition of matter comprising a substantially pure preparation of a flagella-less strain of such a microorganism, and a composition of matter comprising a purified preparation of antigens derived from a culture of a flagella-less strain of such a microorganism.

Brief Summary Text (16):
Also included is an immunoassay procedure for detection of Borreliosis, i.e., infection with borreliae, comprising obtaining a biological sample, such as a sample of a bodily fluid, such as blood, serum, plasma, urine, or synovial or cerebrospinal fluid, from an individual to be tested, contacting the sample with an antigenic preparation derived from a culture of a selected flagella-less strain of borreliae under conditions suitable to allow binding between the antigens in the preparation and borreliae-reactive antibodies in the sample and detecting the binding.

Brief Summary Text (17):
So called "agglutination" assays may be used in accordance with the present invention. In one example, a "latex agglutination" assay, the antigenic preparation is adsorbed or chemically coupled to a particle, such as a latex bead, and particles bearing the antigen are agglutinated under conditions which allow crosslinking of antigen molecules on discrete particles by antibody-antigen complex formation. Alternatively, the antigenic preparations can be used in a so called "microagglutination" or "flocculation" assay, where clumps of antigen-antibody complexes are observed directly.

Brief Summary Text (18):
In another embodiment, the immunoassay may comprise what is known to those of skill in the art as a competitive immunoassay; in such an assay binding is detected by adding a preparation of labeled antibodies reactive with a selected flagella-less Borrelia strain to the contacted sample and measuring binding of the labeled antibody to the antigenic preparation. Because antibodies in the sample will compete with the labeled antibodies for antigenic epitopes in the antigen preparation, binding of the labeled antibody will be inversely proportional to the concentration of antibody in the sample.

Brief Summary Text (19):
Alternatively, the immunoassay procedure may be an immunoassay wherein the binding is detected by adding to the contacted sample a preparation of labeled antibodies that are capable of binding to the antibodies in the sample (e.g., anti-immunoglobulin antibodies) under conditions suitable to allow binding between the labeled antibodies and the antibodies in the samples and measuring the amount of the labeled antibody bound to the antigen-bound antibodies.

Brief Summary Text (21):
The invention also includes procedures for detecting antibodies capable of binding to

immobilizing an antigenic preparation derived from a culture of a flagella-less strain of the Borrelia species on a solid matrix, contacting the immobilized preparation with a sample to be tested for the presence of the antibodies under conditions suitable to allow binding between the antigens in the preparation and the antibodies, separating antibodies bound to the immobilized antigens from the remainder of the sample, and detecting the antigen-bound antibodies. Also included is an additional solid-phase immunoassay for detecting antibodies capable of binding to non-flagellar antigens of a selected Borrelia species comprising obtaining a sample from an individual to be tested, immobilizing antibodies present in the sample on a solid matrix, separating the immobilized antibodies from the sample, contacting the immobilized antibody with an antigenic preparation derived from a flagella-less strain of the selected Borrelia species under conditions suitable to allow binding between the antigens in the preparation and the antibodies, and detecting the antigen-bound antibodies. With this method, the antibodies are detected by contacting the antigen-bound antibody with a detectably labeled antibody capable of specifically binding to the antigen-bound antibody under conditions suitable to allow the binding to occur.

Brief Summary Text (23):
The invention also includes a number of kits for immunoassay. Such kits may comprise, for example, a carrier compartmentalized to contain one or more containers, and a first container containing an antigenic preparation derived from a flagella-less strain of a selected Borrelia species. In one embodiment, the antigenic preparation may be provided immobilized on a solid phase, such as a microtiter well or latex bead. The kit may further comprise a second container comprising a preparation of antibodies reactive with the antigens in the antigenic preparation, and/or a third container containing a detection reagent.

Brief Summary Text (24):
The invention includes vaccines for Borreliosis and vaccination procedures. Vaccines according to the present invention may comprise, for example, an antigenic component derived from a culture of a flagella-less Borrelia strain and a pharmaceutically acceptable carrier. As with other aspects of the invention, the vaccine can comprise any of a number of selected Borrelia species, including but not limited to B. coriacei, for prevention of epidemic bovine abortion; B. recurrentis, B. hermsii, B. turicatae, B. duttoni, B. persica, and B. hispanica, for prevention of relapsing fever; and B. anserina for prevention of avian spirochetosis. Preferably, however, the vaccine will be preventative of Lyme disease in which case it will include an antigenic component derived from a flagella-less strain of B. burgdorferi.

Brief Summary Text (25):
The invention also includes a method for inducing an immune response of a mammal or bird to a microorganism belonging to the genus Borrelia comprising administering an immunogenic dose of the vaccine to the mammal or bird. As those of skill in the art will appreciate, a number of mammals are infected with or are carriers of Borrelia pathogens and thus the invention is not limited by a particular mammal to be injected with the vaccine derived from a particular Borrelia species. However, preferred combinations are those most likely to elicit control or prevention of a commercially significant pathogen. Consequently, vaccines comprising antigens from B. burgdorferi will usually be administered to the primary victims or carriers of Lyme disease such as humans, dogs, horses, equids, cattle, [illegible] victims', deer and rodents, particularly mice (carriers). Vaccines comprising antigens derived from B. coriaceae are usually administered to cattle and those comprising B. anserina, to birds, particularly poultry. Vaccines comprising B. recurrentis and other Borrelia pathogens causing relapsing fever are usually administered to humans.

Brief Summary Text (26):
In addition to the reduced potential for elicitation of undesirable autoimmune responses and the ability to be administered as live attenuated vaccines, the novel vaccines possess the additional advantage of facilitating diagnosis of Borreliosis in individuals, mammals, and birds who have been administered the vaccine. As those of skill in the art will recognize, few if any vaccines are one hundred percent

diagnosis of Borreliosis, and their presence will be suggestive of a vaccine failure.

Drawing Description Text (6):
FIG. 5: Comparison of W, M, and R cells of Borrelia burgdorferi strain HB19 in an ELISA with Lyme disease patient and control sera. The x and y axes are the absorbance values from the assays. Each point denotes the result of each serum in the pairwise comparison. When absorbance results with sera were .gtoreq.1.500 with either of the antigens in the comparison, a single point is shown.

Drawing Description Text (18):
Generally, for detection of antibody in biologic samples, such as blood, plasma, serum, cerebrospinal fluid, synovial fluid, urine and the like, the flagella-less borreliae as antigen, or an antigenic composition prepared therefrom, is preferably adsorbed, or otherwise attached, to an appropriate adsorption matrix (for example, the inside surface of a microtiter dish or well) and a sample of a suspected antibody-containing composition is contacted therewith to cause formation of an immunocomplex between antigens in the composition and any antibodies in the sample that bind to those antigens. The matrix is then washed to remove non-specifically bound material and the immunocomplexes are detected, typically through the use of an appropriate labeled ligand.

Drawing Description Text (19):
Antigen compositions comprising flagella-less borreliae may also be incorporated into diagnostic kits useful in performing assays of the type described above. A number of kits might be utilized in the practice of the present invention, for example, a kit comprising a carrier compartmentalized to contain at least one, at least two, or at least three or more containers.

Drawing Description Text (20):
A first container may include a composition comprising an antigen preparation of the flagella-less borreliae, which may include whole cell preparations or lysates of flagella-less microorganisms or preparations including partially or substantially purified antigenic components derived therefrom, and in particular, cell surface protein antigens. The kits may also include antibody compositions having specificity for one or more Borrelia antigens. Both antibody and antigen preparations should preferably be provided in a suitable titrated form, with antigen concentrations and/or antibody titers given for easy reference in quantitative applications, although the antigenic preparation may also be provided immobilized on a solid matrix.

Drawing Description Text (21):
The kits may also include an immunodetection reagent or label for the detection of specific immunoreaction between the provided antigen and/or antibody, as the case may be, and the diagnostic sample. Suitable detection reagents are well known in the art as exemplified by radioactive, enzymatic or otherwise chromogenic ligands, which are typically employed in association with the antigen and/or antibody, or in association with a second antibody having specificity for the antigen or first antibody. Thus, the reaction is detected or quantified by means of detecting or quantifying the label. Immunodetection reagents and processes suitable for application in connection with the novel compositions of the present invention are generally well known in the art.

Drawing Description Text (22):
The flagella-less borreliae of the invention may also be effectively used as vaccines to prevent Borreliosis, and Lyme disease in particular. In general, immunogenic compositions suitable for administration as vaccines would be formulated to include the flagella-less borreliae, whole cell lysates thereof, or purified antigenic preparations derived from the flagella-less borreliae. Typically, such vaccines are prepared as injectables, either as liquid solutions or suspensions, although solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The preparations may also be emulsified. The reactive immunogenic ingredient is often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol,

will be therapeutically effective and immunogenic. The vaccines may be conventionally administered parenterally, by injection, for example, either subcutaneously or intramuscularly. Where a live vaccine is used, preferred modes of administration are subcutaneous and intradermal injection. Additional formulations which are suitable for other modes of administration may include oral or intranasal formulations. The quantity to be administered will depend on the subject to be treated, capacity of the immune system to synthesize antibodies, and the degree of protection desired. Precise amounts of active ingredient required to be administered will depend on the judgment of the practitioner and may be peculiar to each individual. However, suitable dosage ranges may be on the order of 0.01 ug to 10 mg, and more preferably 1 to 100 ug active ingredient per kilogram of body weight. Suitable regimes for initial administration and booster shots will also be variable, but may be typified by an initial administration followed by subsequent inoculations or other administrations.

Drawing Description Text (24):
In many instances, it may be desirable to have multiple administrations of the vaccine, at from two to twelve week intervals, more usually from three to five week intervals. Periodic boosters at intervals of 1-5 years, usually three years, will be desirable to maintain protective levels of the antibodies. The course of the immunization may be followed by assays for antibodies for the antigens as described above.

Detailed Description Text (19):
SDS-PAGE revealed that the M cells, when compared to W and R cells, lacked a major protein with an apparent molecular weight (M.sub.r) of 37 kDa. No other differences between the proteins profiles of W, M, and R were noted by SDS-PAGE.

Detailed Description Text (19):
The identity of the 37 kDa protein with flagellin was confirmed by Western blot analysis with the monoclonal antibodies H9724 and H604. Monoclonal antibody H9724 binds to native and denatured flagellins of different Borrelia spp. (Barbour et al., 1996). Murine monoclonal antibody H605 is directed against the flagellin of Borrelia burgdorferi (Barbour et al., 1995).

Detailed Description Text (20):
Even with long exposures, full length or truncated flagellin protein was not detectable. Western blots with polyclonal rabbit antisera to Borrelia burgdorferi and Lyme disease patent sera confirmed that M cells appeared to differ from W and R cells only in the absence of a major antigen of approximately 37 kDa (data not shown).

Detailed Description Text (21):
Although the absence of flagellin within the cells of the non-motile mutant was probably the result of failure of expression of the flagellin protein itself, another possible explanation of the findings is that flagellin was produced by the cells but it was not anchored to the hook proteins, and hence lost to the medium. This seemed unlikely, because even transiently associated flagellin should have been detectable in the cells by Western blot. Nevertheless, this alternative explanation was tested by intrinsically labeling the different isolates with .sup.35 S-methionine during growth and then examining the supernatant for evidence of immunoreactive flagellin polypeptides or peptide fragments by immunoprecipitation. The study did not detect the [illegible] flagellin [illegible] in medium [illegible] the flagella less mutant than W and R [illegible] (data not shown).

Detailed Description Text (22):
Antibodies to flagellin have been reported to be a prominent part of the antibody response in early and late Lyme disease (Barbour et al., 1983a; Coleman and Benach 1987; Craft et al., 1986; Grodzicki and Steere, 1988; Wilske, et al., 1988). We compared whole cells of W, M, and R to assess the contribution of antibodies to flagellin in a standard immunologic assay for anti-Borrelia burgdorferi antibodies. The aim of the study was to determine whether the total amount of antibody bound would be detectably lower when the flagella less mutant was used as an antigen than when its flagella-bearing counterparts were used. Sera from patients and controls were examined

The enzyme-linked immunoabsorbent assay (ELISA) using whole cells of Borrelia burgdorferi was a modification of the method of Magnarelli et al. (Magnarelli and Anderson, 1988). Harvested borreliae were suspended in PBS/Mg and an estimate of total cellular protein in the suspension was made using the Bradford assay (Bio-Rad Laboratories, Richmond, Calif.).

Detailed Description Text (33):
The suspension was diluted 1:1000 in a volume of carbonate buffer (Magnarelli and Anderson, 1988) that gave a protein concentration of 1.4 mg/ml; 50 ul of the diluted cell suspension was added to each well of a flat-bottomed, polystyrene microtiter plate (Corning). After incubation of the plates for 18 hrs at 37.degree. C., 200 ul of 1% (w/v) dried nonfat milk in PBS was added to each dry well. Plates were incubated for 1 hour at 37.degree. C. and washed 4 times with 200 ul of PBS. The plates were incubated for 1 hour at 37.degree. C., and then washed with PBS. Bound IgG antibody was assayed with horseradish peroxidase-conjugated, anti-human IgG (gamma-chain specific) goat antisera (Cal-Biochem, San Diego, Calif.) in 1% nonfat milk/PBS buffer. After incubation for 1 hr. at 37.degree. C., the plates were washed 4 times with 200 ul of PBS. The substrate for the peroxidase reaction was O-phenylenediamine dihydrochloride, and absorbance values at 490 mM were recorded on a Dynatech ELISA reader (Model #590). The maximum absorbance value read was 1.5000.

Detailed Description Text (34):
The mean absorbance values (.+- standard error) for the 17 patient sera were 1.39 (.+-.0.09) for W, 1.31 (.+-.0.08) for M, and 1.15 (.+-.0.09) for R cells. Analogous determinations for 19 control sera were 0.19 (.+-.0.03), 0.19 (.+-.0.03), and 0.20 (.+-.0.03), respectively. FIG. 5 shows the pairwise comparisons for W, M, and R cells when patient and control sera were used in the ELISA. There was good correlation between W and R cells for patient sera. Furthermore, when control sera were used the 3 isolates gave comparable results. Surprisingly, the flagella-less M cells had an equal or higher absorbance reading for each patient serum when compared with W and R cells. Many of the values with M cells in the ELISA were .gtoreq.1.50, the absorbance reading maximum. When sera were diluted 1:1000 instead of 1:500 consistently higher absorbance values with M cells as compared with W and R cells was observed (data not shown).

Detailed Description Text (44):
From these studies we concluded that flagella less strains could be isolated from Borrelia burgdorferi cultures and maintained in culture. Furthermore, the absence of flagella does not reduce the efficacy of a Borrelia burgdorferi for serologic testing for antibodies to Borrelia burgdorferi in patients with Lyme borreliosis. Indeed, the use of a flagella-less microorganisms in a serologic assay is likely to improve the sensitivity and specificity of the assay. Although applicants are not bound by theory, the possible reasons for this are the following: (a) When flagella are not present, the relative amounts of other proteins in the suspension on a protein concentration basis increases. One or more of these other proteins (or non-proteins) may be more important than flagella for diagnostic purposes. (b) Flagella are likely a major source of false-positive reactions, because of the cross-reactivities between the flagella of Borrelia burgdorferi and those of other bacteria. When flagella are not present, the specificity of the assay may improve. The flagella-less isolate could be used in place of borrelia with the wild-type phenotype in immunofluorescence, ELISA-based, and Western blot assays.

Detailed Description Text (47):
A flagella-less Borrelia burgdorferi may be safer to use as the basis of a whole cell or subunit vaccine for protection against Lyme disease or other Borrelia infections than an antigen preparation containing flagella. Studies have demonstrated cross-reactions between flagellar-associated antigens and human tissues, including nerve and muscle. It is possible that vaccines containing large or small amounts of flagellar materials will induce an autoimmune reaction in the recipient.

Detailed Description Text (51):
FIG. 7 shows the results of this experiment. All rat sera, including the control sera,

Detailed Description Text (53):
In additional studies, rats were immunized intramuscularly with 20 .mu.g total protein of borreliae in complete Freund's adjuvant. After 6 weeks, the rats were bled and their serum was analyzed by ELISA, essentially as described above. The results of this experiment shown in Table 3, indicated that immunization with M cells induced a significant immune response against W cells.

Detailed Description Text (55):
As shown, serum from immunized rats, even at a dilution of 1:3,000 was capable of neutralizing B. burgdorferi. Thus, when used to immunize rats, flagella-less (M) cells were as effective as flagella-bearing (W) cells in eliciting antibodies to B. burgdorferi as determined by ELISA and by growth inhibition assay.

Detailed Description Text (63):
2. A. G. Barbour, Isolation and cultivation of Lyme disease spirochetes, Yale J. Biol. Med. 1984; 57:521-5.

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3. A. G. Barbour et al., Antibodies of patients with Lyme disease to components of the Ixodes dammini spirochete, J. Clin. Invest. (1983a); 72:504-15.

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7. A. G. Barbour et al., Variation in a major surface protein of Lyme disease spirochetes, Infect. Immun. 1984; 45:94-100.

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Detailed Description Text (79):
18. J. L. Coleman and J. L. Benach, Isolation of antigenic components from the Lyme disease spirochete their role in early diagnosis, J. Inf. Dis. 1987; 155:756-765.

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Detailed Description Text (89):
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Detailed Description Text (103):
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Detailed Description Text (110):
49. L. H. Sigal and A. H. Tatum, IgM in the Sera of Patients with lyme Neurologic Disease Bind to Cross-reacting Neuronal and Borrelia burgdorferi Antigens, Ann. NY Acad. Sci. 539:422-424 (1988).

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Detailed Description Text (115):
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Detailed Description Text (116):
55. A. C. Steere, Lyme disease, New Engl. J. Med. 1989; 321:586-596.

Detailed Description Text (118):
57. D. D. Thomas and L. E. Comstock, Interaction of Lyme disease spirochetes with cultured eucaryotic cells, Infect. Immun. 1989; 57:1324-6.

Detailed Description Text (119):
58. R. Wallich et al., The Borrelia burgdorferi flagellum-associated antigen (flagellin): molecular cloning, expression, and amplification of the gene, Infect. Immun. 1990; 58:1711-1719.

Detailed Description Paragraph Table (3):
TABLE 3 ELISA Test With Serum Diluted 1:1,000

Absorbance Rat #	Immunogen	ELISA antigen	Value
1	W cells	W cells	0.713
	M cells		0.727
2	W cells	W cells	0.592
	M cells		0.629
3	M cells	W cells	0.555
	M cells		0.449
4	M cells	W cells	0.589
	M cells		1.082
5	Adjuvant alone	W cells	0.010
	M cells		0.014
6	Adjuvant alone	W cells	0.015
	M cells		0.013

WEST Search History

DATE: Friday, August 30, 2002

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ			
L11	L9 and (37 KD or 37 kda or 37kd or 37kda)	4	L11
L10	L9 and immunoassay	41	L10
L9	L8 and antigen	80	L9
L8	l6 and immunodominant	80	L8
L7	L6 and (p37 or flaa)	13	L7
L6	l4 and L5	839	L6
L5	diagnostic or elisa or detect? or diagnos?	738145	L5
L4	lyme disease	1369	L4
L3	johnson-barbara-j-b.in.	2	L3
L2	gilmore-r-d.in.	2	L2
L1	gilmore-robert-d.in.	0	L1

END OF SEARCH HISTORY